CHARACTERIZING GLUCOCORTICOID LEVELS IN FIVE SPECIES OF SEA DUCKS OCCURING IN ALASKA

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ABSTRACT

Stress hormone analysis, used in conjunction with other physiological parameters, may help identify factors affecting sea duck populations in their natural environment. Corticosterone, the primary "stress hormone" in birds, is secreted in response to a stressor and enhances an individual's chance of survival by inducing physiological and behavioral changes. Establishing a valid method for evaluating stress hormone levels in sea ducks and gaining basic information on baseline concentrations and stress response in these birds are important first steps to identify factors that may negatively affect sea duck populations.

This study validated a radioimmunoassay (RIA) procedure to measure corticosterone concentrations in harlequin duck serum and feces and in Steller's, spectacled, common, and king eider serum. Other objectives included characterization of baseline corticosterone concentrations, investigation of stress response, and the relationship between corticosterone and other variables in captive and wild sea ducks.

The results indicate that fecal samples can be used to non-invasively measure corticosterone in harlequin ducks. Captive birds exhibited overall lower baseline levels of corticosterone than wild birds. The stress response observed in harlequin ducks was similar to other avian species. Rapid post-capture blood sampling is critical for evaluation of baseline corticosterone levels.

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GENERAL INTRODUCTION

The tribe *Mergini* or sea ducks is a diverse group of birds consisting of 18 extant and two extinct species. Members of this tribe typically inhabit temperate to sub-polar areas in the northern hemisphere with only one extant species occurring in the southern hemisphere, the Brazilian merganser (*Mergus octosetaceus*). Sea ducks generally exhibit similar traits such as sexual dimorphism (Batt *et al.,* 1992), delayed breeding until 2-3 years of age (Bellrose, 1980), relatively long life spans (up to 20 years for some species; Bellrose, 1980), and low reproductive success (Sea Duck Joint Venture Management Board, 2001).

There are still gaps in the biological knowledge of sea ducks as a group. Due to the remote locations that sea ducks generally inhabit, basic life history parameters are missing for some species. Population estimates and trends are difficult to obtain and are often based on a small number of studies. Furthermore, identification of threats to populations is often time and cost intensive (Sea Duck Joint Venture Management Board, 2001). However, several populations of sea ducks in North America are known to have declined over the past two to three decades and the causes are unclear for most species (Stehn *et al.*, 1993; Henny *et al.,* 1995; Suydam et al., 2000; Sea Duck Joint Venture Management Board, 2001). Sea ducks may be especially sensitive to anthropogenic disturbances (e.g., contaminants, hunting, and loss of habitat) and if these types of disturbances become more prevalent in their natural environment, further population declines may occur (Henny *et al.*, 1995; Irons *et al.*, 2000; Wentworth and Wong, 2001). The following sections discuss distribution, abundance, status, and current threats for the species in the present study.

Harlequin duck (*Histrionicus histrionicus*)

Distribution: Four separate populations of harlequin ducks are recognized based on breeding sites: Iceland, Greenland, and the eastern and western North American populations (Bellrose, 1980). The populations in Greenland and Iceland breed, molt, and winter in these regions (Gooders and Boyer, 1986). The western North American population of harlequin ducks breeds along streams in Alaska, British Columbia, the northwestern contiguous United States, the Rocky Mountains, and northeastern Russia. This population winters along the coasts of Russia, Alaska, and British Columbia (Bellrose, 1980; Gooders and Boyer, 1986; Sea Duck Joint Venture Management Board, 2001). Harlequin ducks belonging to the eastern North American population breed in eastern Canada, and winter along the coasts of the northeastern United States and eastern Canada (Bellrose, 1980; Gooders and Boyer, 1986). In addition, some birds from this population migrate to Greenland to winter (Boertman and Mosbech, 2002; Gilliland *et al.*, 2002).

World Population: The current population estimate for the eastern population is 2,000 birds, whereas the status of the western population is uncertain (Sea Duck Joint Venture Management Board, 2001), although it is believed to number in the low hundreds of thousands (Dan Esler pers. comm.). No accurate population estimates are available for the populations breeding in Greenland and Iceland.

Abundance and status: The eastern North American population has been given an "endangered" status in Canada (Montevecchi *et al.,* 1995).

Threats: An estimated 1,300 harlequin ducks died as a direct result of the *Exxon Valdez* oil spill in Prince William Sound (PWS), Alaska, in 1989 (US Fish & Wildlife Service, 1999). The continued negative effects of the oil spill on harlequin duck numbers in PWS was still pronounced several years after the spill and has prevented the population from recovering (Irons *et al.,* 2000; Esler *et al.*, 2000; Trust *et al.*, 2000, Esler *et al.,* 2002). Irons *et al.* (2000) found

strong negative effects on population density of harlequin ducks in oiled areas of PWS one to two years after the *Exxon Valdez* oil spill. Esler *et al.* (2000a) reported "considerably higher" densities of harlequin ducks at un-oiled Montague Island compared to oiled Knight Island several years after the oil spill. Residual oil continued to affect harlequin duck populations in PWS nine years after the oil spill (Esler *et al.*, 2002). Exposure to oil continued during 1998, resulting in lower adult female survival in areas where residual oil was present, as well as overall lower population numbers (Trust *et al.*, 2000, Esler *et al.,* 2002). Demographic studies by Lanctot *et al.* (1999) suggest that population recovery through emigration into areas of PWS where populations decreased as a result of the oil spill may take a long time due to low rates of movement between populations. Known predators of harlequin ducks include eagles, hawks, owls, mink, and river otters (Dan Esler pers. comm.). Harbor seal (*Phoca vitulina*) predation on a single harlequin duck was reported by Tallman and Sullivan (2004). Although not a major sport or subsistence hunting target, an estimated 500 to 1,000 harlequin ducks are taken annually in Alaska (Sea Duck Joint Venture Management Board, 2001).

Steller's eider (*Polysticta stelleri)*

Distribution: This species breeds in Arctic Russia and Alaska, with the majority of individuals belonging to two recognized Russian populations (Bellrose 1980, Gooders and Boyers 1986). In Alaska, Steller's eiders breed along the North Slope and, historically, bred regularly in the Yukon-Kuskokwim (Y-K) Delta (Fredrickson, 2001). Steller's eiders winter in the eastern Aleutian Islands, along the coast of the Alaska Peninsula, and the coastal waters off south central Alaska (Fredrickson, 2001). Russian populations winter in Alaska, arctic Europe, and in both northeast and northwest Russia (Systad and Bustnes, 2001; Krasnov and Goryarev, 2001). Steller's eiders primarily molt in lagoons along the Alaska Peninsula and northwest Eurasia

(Petersen, 1981; Gooders and Boyer, 1986). Steller's eiders separate spatially, and to some extent temporally, according to sex and age classes during molting (Petersen, 1981).

World population: The current worldwide population is estimated at 130,000-150,000 birds with over 100,000 individuals in the Pacific population and 30,000-50,000 birds wintering in northern Europe (Sea Duck Joint Venture Management Board, 2001).

Abundance and status: The Alaskan breeding population was listed as "threatened" under the Endangered Species Act in 1997, because of its decrease in numbers and range (Federal Register, 1997). Between the late 1970's and the early 1990's a slight decline in annual adult survival rate due to unknown causes was reported by Flint *et al.* (2000), which could potentially be a factor in the overall population decline observed in the past two decades.

Threats: Flint *et al.* (2000) found lower annual survival rates for males than females, which may result in a female-biased sex ratio. This could limit the reproductive potential of this monogamous species (Bellrose 1980). Dau *et al.* (2000) found no evidence to support the hypothesis that the differential harvest of male and female birds by native hunters was causing the observed difference in survival rates. Arctic foxes (*Alopex lagopus*), pomarine jaegers (*Stercorarius pomarinus*), and ravens (*Corvus corax*) are known to prey on Steller's eider eggs, whereas snowy owls (*Nyctea scandiaca*), peregrine falcons (*Falco peregrinus*), and gyr falcons (*Falco rusticolus*) have been observed preying on adults (Quakenbush and Suydam, 1999).

Spectacled eider *(Somateria fischeri)*

Distribution: Spectacled eiders breed in western and northern Alaska (i.e., the Y-K Delta and the North Slope/Arctic Coastal Plain) and on the coastal tundra of Siberia (Grand and Flint 1997; Lovvorn *et al.*, 1997; Gooders and Boyers, 1986). Molting occurs along the Chukotsk coast, Russia, along the coast of western Alaska, and offshore south of St. Lawrence Island in the

Bering Sea (Larned *et al.*, 1995). The entire world population is thought to winter in ice leads south of St. Lawrence Island in the Bering Sea (Petersen *et al.*, 1999).

World population: The wintering population in the Bering Sea, which is thought to represent the entire population, has been estimated at 363,000 birds (Larned and Tiplady, 1999).

Abundance and status: Stehn *et al.* (1993) reported a decline of 94% (from 48,000 pairs to fewer than 5,000 pairs) in the breeding population in the Y-K Delta between the 1970s and 1992. This rapid decline prompted the listing of the spectacled eider as "threatened" under the Endangered Species Act in 1993 (Federal Register, 1993).

Threats: Several studies have shown that poisoning from the ingestion of lead shot is a cause of mortality in spectacled eiders breeding in the Y-K Delta (Franson *et al.*, 1995; Flint *et al.*, 1997; Grand *et al.*, 1998). According to Flint *et al.* (1997), 25-37% of the breeding population in the Y-K Delta has been exposed to lead. Grand *et al.* (1998) estimated that approximately 29% of annual mortality of spectacled eiders breeding along the Kashunuk River (Y-K Delta) is due to lead exposure. Glaucous gulls (*Larus hyperborus*), parasitic jaegers (*Stercorarius parasiticus*), Artic foxes, and red foxes (*Vulpes vulpes*) are potential predators of spectacled eider nests (Dau, 1974). Furthermore, mew gull (*Larus canus*) predation of ducklings has been suggested as a cause of nest failure (Grand and Flint, 1997). Spectacled eiders are harvested in small numbers in the Y-K Delta, with an average estimated annual take of 170 birds between 1990 and 1999 (Wentworth and Wong, 2001). The annual take estimates decreased by two thirds over this nine year period (Wentworth and Wong, 2001). Researchers found antibodies to the infectious bursal disease virus (IBDV) in spectacled eiders nesting in western Alaska, indicating the presence of this virus in this population (Hollmén *et al.*, 2000). This finding raised concerns, because IBDV has been shown to cause up to 30% mortality in juvenile poultry (Lukert and Saif, 1997).

Common eider (*Somateria mollissima*)

Distribution: There are six recognized sub-species or races of the common eider: 1) the European race (*Somateria mollissima mollissima*), 2) the southern race (*Somateria mollissima dresseri*), 3) the Pacific race (*Somateria mollissima v-nigra*), 4) the Hudson Bay race (*Somateria mollissima sedentaria*), 5) the Faeroe race (*Somateria mollissima faeroeensis*), and 6) the northern race (*Somateria mollissima borealis)* (Gooders and Boyer, 1986). The common eider is a pan-arctic species with breeding areas in Alaska, Canada, Greenland, Iceland, Scandinavia, the British Isles, Russia, and the Netherlands, and wintering areas extending further south to include France and Spain, southwest Alaska, British Columbia, the American northeastern and eastern states and the Kamtchatka Peninsula in Russia (Bellrose, 1980; Gooders and Boyer, 1986).

World population: The world population of common eiders has been estimated at over two million birds (Circumpolar Seabird Working Group, 1997).

Abundance and status: Several common eider races (e.g., the Pacific and the Hudson Bay race) have declined during the 1980's and 1990's (Sea Duck Joint Venture Management Board, 2001). Suydam *et al.* (2000) report a 53% decline in Pacific common eiders migrating past Point Barrow, Alaska between 1976 and 1996.

Threats: Common eider mortality due to ingestion of spent lead shot has been reported (Franson *et al.*, 1995). Herring gulls *(Larus argentatus*) were reported to take up to 30% of the eggs laid in a common eider colony on Southampton Island, Canada (Allard and Gilchrist, 2002). Other nest predators at this site included polar bears (*Ursus maritimus*) and Arctic foxes (Allard and Gillchrist, 2002). Subsistence hunting for common eiders in Alaska and Canada can be locally important for native peoples. The annual harvest of northern common eiders in Greenland can be in excess of 100,000 individuals (Sea Duck Joint Venture Management Board, 2001).

Hollmén *et al.* (2000) found antibodies to IBDV and identified reoviruses in common eiders breeding in the Baltic Sea, and the presence of these viruses could potentially explain the poor fledgling success in common eiders nesting in the Baltic Sea. It has been suggested that starvation resulting from over-harvesting of cockles and mussels caused a mass die-off of 21,000 common eiders wintering in the Dutch Wadden Sea (Camphuysen *et al.,* 2002). Hatching probability decreased from 41% in the year before the die-off, to 18% in the subsequent breeding season among eiders nesting on the island of Griend, Wadden Sea (Oosterhuis and van Dijk, 2002). These findings suggest a link between nutritional deficiencies and decreased fecundity in this species (Oosterhuis and van Dijk, 2002).

King eider (*Somateria spectabilis*)

Distribution: King eiders breed in the Canadian Arctic, Greenland, Alaska, and Russia (U.S. Fish and Wildlife Service, 1999; Sittler *et al.,* 2000; Kellett *et al.*, 2003). Wintering king eiders are found in eastern Canada (Labrador and Newfoundland), northern Alaska, the Bering Sea, and Greenland (Sea Duck Joint Venture Management Board, 2001).

World population: The world population is estimated to number at least one million individuals (Circumpolar Seabird Working Group, 1997).

Abundance and status: Suydam *et al.* (2000) reported a 56% decline in king eider numbers migrating past Point Barrow, Alaska between 1976 and 1996, indicating a drastic population decline in this area. Overall, the population of king eiders breeding in Canada is believed to be decreasing (U.S. Fish & Wildlife Service, 1999; Circumpolar Seabird Working Group, 1997). The status and trends for king eiders in Russia are currently unknown (Circumpolar Seabird Working Group, 1997).

Threats: Elevated concentrations of contaminants known to have severely adverse health effects, (e.g., lead, copper, and selenium) have been found in livers, kidneys, and muscle tissue samples of king eiders (Stout *et al.,* 2002; Savinov *et al.*, 2003). As with many other species of marine birds, oil spill induced mortality has been observed in king eiders. Flint *et al.* (1999) estimated that 1600 dead king eiders were washed ashore on St. Paul Island, Alaska after the M/V *Citrus* oil spill in the Bering Sea in 1996. Kellet *et al.* (2003) found that predation accounted for 66% of nest failures for king eiders breeding in Nunavut, Canada. Although no predation event was observed, the authors suspected that arctic foxes, jaegers (*Stecorarius spp.*), and gulls (*Larus spp.*) were responsible for the majority of nest losses due to their abundance in the area (Kellet *et al.*, 2003). In Greenland, collared lemming (*Dicrostonyx groenlandicus*) abundance is typically highly variable from year to year and some predators prey mainly on lemmings during years of high lemming densities thereby lowering predation pressure on alternate prey (Sittler *et al.*, 2000). It has been suggested that increased nest predation during years with low lemming densities was responsible for the observed low rates of king eider breeding success in northeast Greenland (Sittler *et al.*, 2000). An observed spatial and temporal nest association between king eiders and long-tailed jaegers (*Stercorarius longicaudis*) breeding in northeastern Greenland is believed to be a strategy against nest predation; long-tailed jaegers defend their nests, and inadvertently, the nests of birds breeding nearby, against predators (Blomquist and Elander, 1988). Sport and subsistence hunting for king eiders occurs throughout much of its range. In Greenland, the reported annual harvest of king eiders is about 5000 individuals and the actual number is believed to be considerably higher (Circumpolar Seabird Working Group, 1997). The average annual harvest of king eiders in the Y-K Delta in Alaska was 2900 ducks between 1990 and 1999 (Wentworth and Wong, 2001).

Stress hormones

Physiology of the stress response

A stressor is any threat or event, real or perceived, which has the potential to alter an organism's homeostasis or challenge its survival. When subjected to a stressor the neuroendocrine system activates a cascade of biochemical reactions which enable survival-enhancing changes in an organism's physiology (Harvey *et al.*, 1984). The stress response primarily occurs in the hypothalamo-pituitary-adrenocortical (HPA) axis. Neural stimuli triggered by an exogenous stressor cause the release of corticotropic releasing factors (CRF) by the hypothalamus. This process, in turn, facilitates the production of adrenocorticotropic hormone (ACTH) by the pituitary gland and its subsequent release into the blood stream. ACTH causes the release of glucocorticoids (GCs; cortisol and corticosterone) by the adrenal gland (Norris, 1997; Norman and Litwack, 1998). Corticosterone (CORT) is the primary glucocorticoid found in birds (Wingfield *et al.,* 1994a; Astheimer, 1995; Silverin, 1997; Wingfield *et al.*, 1998).

Physiological and behavioral effects of corticosterone

The release of GCs induces survival-enhancing physiological and behavioral changes in an organism (Wingfield *et al.,* 1998). When an organism responds to an external stressor, functions directly related to immediate survival, such as the mobilization of energy (gluconeogenesis), are enhanced, and non-essential processes are inhibited or temporarily suspended (McEwen and Stellar, 1993; Wingfield *et al.*, 1998). Behavioral effects directly linked to elevated GC concentrations include hyperphagia (Koch *et al.*, 2001), suspended breeding (Astheimer, 1995; Silverin, 1998b), increased begging by chicks (Kitaysky *et al.,* 2003), and dispersal (Heath, 1997; Silverin, 1997). The physiological and behavioral effects of a short term increase in CORT concentrations (i.e., "rapid" or "acute" stress response) are considered adaptive

and beneficial, whereas long-term exposure to elevated GC concentrations (i.e., "chronic" stress) can be devastating to an organism (Wingfield *et al.*, 1998). Chronically elevated CORT concentrations cause suppression of the immune and reproductive systems (Wingfield *et al.*, 1998), gastrointestinal dysfunction (Monnikes *et al.*, 1994), and eventually death (Wingfield *et al.,* 1998). A rapid adrenocortical stress response may help an individual avoid a state of chronic stress by temporarily altering its physiology and behavior (Wingfield *et al.*, 1998; Breuner *et al.*, 1999), and the hormones produced at this stage may be more appropriately called "anti-stress hormones" (Wingfield and Kitaysky, 2002).

Baseline concentrations versus stress response

It is important to differentiate between baseline CORT concentrations, and CORT concentrations caused by the physiological response to a stressor, that is, the stress response (Silverin, 1998a). Both baseline CORT concentrations and the magnitude of the stress response typically fluctuate on a daily (Wilson *et al.*, 1982; Westerhof *et al.*, 1994; Breuner *et al.,* 1999), seasonal (Wingfield *et al.*, 1994b; Romero *et al.,* 1998; O'Reilly and Wingfield, 2003), and annual basis (Mizrahi *et al.,* 2001). The stress response, and its consequent effects on physiology and behavior, has been found to differ between gender (Astheimer *et al.,* 1994) and age class in several bird species (Silverin, 1997; Sims and Holberton, 2000). There is also substantial individual variation (Vleck, 2000; Cockrem and Silverin, 2002a), as well as intraspecific differences with breeding locale (Romero *et al.*, 1998; Silverin and Wingfield, 1998).

Species (or populations within a species) breeding in "harsh" or demanding environments, such as high latitudes or deserts, typically show high baseline concentrations and a suppressed stress response compared to similar species or populations breeding in more benign environments (Silverin and Wingfield, 1998; Wingfield *et al.*, 1992). Silverin *et al.* (1997)

compared the stress response in two populations of willow warblers (*Phylloscopus trochillus*), one breeding in a sub-arctic environment in northern Sweden (i.e., a "harsh" environment) and another breeding in a more temperate climate in southern Sweden. The authors found significantly decreased stress responses in both sexes in the warblers breeding in the more severe climate of northern Sweden, suggesting that these birds were more resistant to acute stress. These birds bred under unpredictable climatic conditions with a very short breeding season. Silverin *et al.* (1997) argue that this increased resistance to stress is highly adaptive since it allows the northern breeding birds to continue breeding in spite of adverse physical conditions, which would have caused an interruption of breeding had their stress response been the same as the southern breeding birds.

Migratory bird species typically show low baseline concentrations of CORT and a strong stress response when not migrating, and high baseline CORT concentrations with a suppressed stress response during migration (Holberton *et al.*, 1996). Suppression of the stress response during migration is believed to be a protective measure against the breakdown of muscle tissue as a consequence of catabolic processes (i.e., gluconeogenesis) induced by elevated circulating concentrations of CORT (Holberton *et al.,* 1996). Ramenofsky *et al.* (1995) reported an absence of a stress response in adult bar-tailed godwits (*Limosa lapponica*) at a stopover site during spring migration. Furthermore, the authors found an inverse relationship between body condition and CORT concentrations, as well as a decrease in baseline concentrations as body mass increased during the one month staging period. Mizrahi *et al.* (2001) found a suppressed stress response in migrating semi-palmated sandpipers (*Calidris pusilla*) that showed low rates of mass gain or were in relatively poor condition. The authors suggest that this may be explained by the necessity to protect skeletal muscles needed for flight from the catabolic properties of CORT.

Natural and anthropogenic causes of stress in birds

An organism is exposed to a host of natural and anthropogenic stressors in its environment. Natural stressors include predators, inclement weather, and shifting food supply (Smith *et al.*, 1994; Kitaysky, 2001; Cockrem and Silverin, 2002b), whereas anthropogenic stressors include factors such as petroleum fouling through oil spills, human encroachment, and exposure to environmental contaminants such as heavy metals (Holmes *et al.,* 1979; Fowler, 1999; Ruiz *et al.,* 2002).

The effects of the presence of a predator on CORT concentrations in birds were demonstrated by Cockrem and Silverin (2002b) who found significantly increased plasma CORT concentrations in great tits (*Parus major*) in response to a stuffed owl. In addition, the authors exposed the birds to two other unknown objects (a stuffed brambling and a cardboard box). These items failed to elicit a response, indicating that the observed response to the owl was specifically related to its predation potential. Silverin (1998b) reported increased CORT concentrations in male pied flycatchers (*Ficedula hypoleuca*) as a response to a model of a weasel, which is a common predator of this species.

Adélie penguins (*Pygoscelis adeliae*) are adapted to fasting during incubation. Vleck *et al.* (2000) found no evidence of an increase in baseline CORT concentrations during a fast of normal length (up to 40 days) in this species. However, individuals fasting much longer than the norm (e.g., 55 days) exhibited significantly elevated baseline CORT concentrations. Hood *et al.* (1998) found an increasing stress response in Magellanic penguins (*Sphenicus magellanicus*) as fasting depleted their fat stores. Kitaysky *et al.* (2001) showed that food deprivation caused a long-term elevation of baseline CORT concentrations in red-legged kittiwake chicks (*Rissa brevirostris*) and suggested that this type of stressor might induce a state of chronic stress.

Kitaysky *et al.* (1999) reported that dietary restrictions resulted in similar effects in black-legged kittiwake (*Rissa tridactyla*) chicks.

The effects of inclement weather on baseline concentrations of CORT have been studied in the common diving petrel (*Pelecanoides urinatrix*) (Smith *et al.*, 1994). Significantly elevated baseline concentrations of CORT were found during a severe storm. There was no increase in baseline concentrations of CORT after one hour of handling, suggesting that the birds were maximally stressed when captured (Smith *et al.,* 1994).

Ruiz *et al.* (2002) found that rufous-collared sparrows (*Zonotrichia capensis*) living in urban areas in Chile had higher CORT concentrations, lower body weight, and higher blood glucose concentrations than rural con-specifics, which suggested that urban birds were in a state of chronic stress. Rural birds kept in captivity for two weeks developed blood indices resembling those of the urban sparrows (Ruiz *et al.*, 2002).

Oil ingestion was found to be a non-specific stressor in captive mallards (*Anas plathyrhynchos*) which predisposed birds to the harmful effects of chronically elevated CORT concentrations (Holmes *et al.,* 1979). Birds fed oil-contaminated food exhibited adrenal hypertrophy and were more likely to die from cold stress than non-treated birds (Holmes *et al.,* 1979). Fowler *et al.* (1995) found elevated CORT concentrations in female Magellanic penguins exposed externally to low concentrations of petroleum contamination; however, this correlation was not observed in males.

Stress hormone concentrations as an indicator of the health of a population

Stress hormone concentrations have been suggested to be "a powerful predictor for the rapid assessment of wildlife health" (Romero and Wikelski, 2002). CORT concentrations could be used as an indicator of overall population health, where elevated baseline CORT

concentrations may indicate a struggling population. Studies on a variety of bird species have shown a negative correlation between the magnitude of the stress response and the thickness of fat deposits. Birds with higher fat stores exhibited a reduced stress response (Schwabl *et al.*, 1991; Wingfield *et al.*, 1994a; Wingfield *et al.*, 1994b). Such findings suggest a relationship between better overall health, larger fat stores, and a lower stress response. CORT baseline concentrations in conjunction with other measurable parameters (e.g., body condition index and heterophil to lymphocyte ratio) could indicate the health of population– information that would be useful to wildlife managers and policy makers and which could be used to make informed management and conservation decisions.

The overall objectives of this study were to validate the use of a commercially available radioimmunoassay kit to evaluate CORT concentrations in sea ducks and to characterize adrenal function in terms of CORT concentrations in captive and wild harlequin ducks and eiders. Specific objectives included an adrenocorticotropic hormone (ACTH) challenge to determine maximum CORT concentrations, to assess if fecal samples can be used as a non-invasive alternative to serum in measuring CORT in sea ducks, to investigate capture induced stress response, and to determine the relationships between CORT and other variables (e.g. mass and season) in sea ducks.

Chapter 1. Evaluating adrenal function in harlequin ducks (*Histrionicus histrionicus*): focus on stress hormones[1]

INTRODUCTION

Adrenal glucocorticoid (GC) hormones, cortisol and corticosterone (CORT), are secreted by the adrenal cortex and play important roles in gluconeogenesis, mobilization of energy, and growth and development in birds (Cahill, 1971; Thompson and Lippman, 1974; Wingfield *et al.*, 1994a; Norris; 1997). GCs, or "stress" hormones, also influence many other physiological processes that allow an animal to adapt and function in situations that it perceives as stressful (Harvey *et al.*, 1984). CORT has been established as the primary circulatory GC in birds (Holms and Phillips, 1976; Harvey and Phillips, 1982; Wingfield *et al.*, 1992). Several factors have been found to increase CORT concentrations in birds, including insufficient food and water (Rees *et al.*, 1985; Harvey and Hall, 1990; Kontecka *et al.*, 1999), capture and handling (Wingfield *et al.*, 1982; Romero *et al.*, 1997; Gratto-Trevor *et al.*, 1991; Silverin and Wingfield, 1998), exercise (Harvey and Phillips, 1982), social rank (Schwabl *et al.*, 1988; Nunez-de La Mora *et al.*, 1996), and heat stress (Edens and Siegel, 1975). GC increases allow a shift from processes which are non-essential for immediate survival to those that favor rapid response to the environmental change or stressor (Kacsoh, 2000; St. Aubin, 2001). In birds, effects of chronic or prolonged increase in circulating GCs include reduction in size of the ovary (Petite and Etches, 1991) and testes (Gross *et al.*, 1980), growth inhibition (Davison *et al.*, 1980), reduced immune response (Gross *et al.*, 1980), gastrointestinal dysfunction (Monnikes *et al.*, 1994), and increased salt gland activity (Phillips, 1968; Holmes, 1972).

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The media most commonly used to assess CORT concentrations in birds is plasma and serum (Wingfield *et al.*, 1982; Silverin *et al.*, 1993; Wingfield *et al.*, 1994b). Since the capture procedure itself has been shown to constitute a stressor, it is imperative that the blood sample be obtained within minutes of capture in order to get a baseline value of CORT (Wingfield *et al.,* 1982; Romero *et al.,* 1997; Silverin and Wingfield, 1998). For passerines, a sample obtained within 3 minutes is typically considered to represent CORT baseline concentrations whereas a 30 minute sample represents the maximum stress response (Wingfield *et al.,* 1982, Wingfield *et al.*, 1994b, Romero *et al.*, 2000). Several studies have shown that other media (such as feces and cloacal fluids) can be used to assess CORT concentrations in many different bird species (Wasser *et al.*, 1997; Hiebert *et al.*, 2000; Wasser *et al.*, 2000; Goymann *et al.*, 2002). Using feces or cloacal fluids for CORT analysis has the advantage of being less invasive as well as a providing a measurement over a longer period of time (Wasser *et al.*, 2000). As such, fecal samples may be used to investigate baseline concentrations of CORT when it is difficult or impossible to obtain a blood sample within a few minutes of capture.

Injection of exogenous adrenocorticotropic hormone (ACTH) has been used to evaluate adrenal function in several avian taxa (e.g., psittacines: Zenoble *et al.*, 1985; raptors: Zenoble *et al.*, 1985b; waterfowl: Spelman *et al.*, 1995; and cranes: Ludders *et al.*, 1998) by stimulating a physiological stress response characterized by elevated CORT concentrations. The adrenal reaction characterized by maximum CORT output and lag time in response to an ACTH-challenge/stimulation, has not been documented for any sea duck species.

Organisms exhibit persistent physiological rhythms with circadian (daily), tidal or lunar (monthly), and circannual (yearly) periods (Leland and Edmunds, 1988). Basal plasma corticosteroid concentrations fluctuate on seasonal and circannual scales for several bird species (domestic pigeons, *Columbia livia*: Sato and George, 1973; mallards, *Anas platyrhynchos*: Wilson

et al., 1982; white-crowned sparrows, *Zonotrichia leucophrys*: Astheimer *et al.*, 1994; starlings, *Sturnus vulgaris*: Romero and Wingfield, 2000; western sandpipers, *Calidris mauri*: O'Reilly and Wingfield, 2003). Astheimer *et al.* (1994) showed that the circannual rhythm of CORT production was strongly correlated with season in one arctic bird species, the white crowned sparrow. The main environmental cue or *zeitgeber* of GC rhythmicity in bird species studied to date is photoperiod (Daan and Gwinner, 1989). However, information on circadian patterns of corticoid production in sea ducks is lacking in the literature.

The overall objectives of this study were: i) to validate the use of a commercially available radioimmunoassay kit in measuring CORT concentrations in harlequin ducks and, ii) to characterize adrenal function in captive and wild harlequin ducks. Understanding adrenal function in harlequin ducks (e.g., shape of the stress response curve, maximum adrenal output, etc.) will enhance our knowledge of the physiology of the species as well as help us understand the relationship between these birds and their environment. CORT concentrations could be used, in conjunction with other physiological parameters (e.g., body condition indexes, heterophil to lymphocyte ratio, and other hematological measurements), to evaluate the health of a population. For captive harlequin ducks, the goals of this study were: i) to determine baseline circadian patterns of GCs, ii) to establish the maximum output of CORT through an ACTH challenge, iii) to investigate the lag-time between a stressor (exogenous ACTH) and the maximum stress response (peak CORT concentration), iv) to investigate the validity of fecal samples for CORT analysis, and v) to determine the relationship between CORT concentrations and diet, body weight, severity of bumblefoot, packed cell volume, heterophil: lymphocyte ratio, time since capture, restraint/handling time, bleeding site, and percent of blood volume collected.

For wild harlequin ducks, the goals were i) to investigate the effect of capture and short term holding on CORT concentrations and ii) to investigate differences in CORT concentrations

in two populations of harlequin ducks in mid 1990's in Prince William Sound, Alaska: one residing in an area where residual oil from the *Exxon Valdez* oil spill in 1989 was still present during the sample collection and another where no oil was present.

MATERIALS AND METHODS

Captive harlequin ducks

Harlequin ducks ($N = 12$; 11 females, 1 male) were caught in Prince William Sound (PWS), Alaska (60° 45' N; 156° 47' W), in September 2001. The average mass of the birds used in this study was 533 g (range: 492 to 613 g). The ducks were housed in outdoor enclosures under ambient conditions at the Alaska SeaLife Center, Seward, Alaska (60° 69' N; 149° 26' W). Each enclosure had a floor space of 8.7 m^2 and a circular saltwater pool with an area of 4.7 m^2. Six birds were housed in each enclosure. Half of the birds were fed a diet of Atlantic silversides (*Menidia menidia*) and the other half received silversides plus Antarctic krill (*Euphausia superba*) with multivitamin supplementation (Tuomi *et al.*, 2003).

Sampling regime

Blood sampling. All blood samples were drawn via jugular, right or left brachial, or tarsal venipuncture utilizing a sterile 1 cc syringe and a 25 gauge needle and refrigerated prior to processing. Blood was centrifuged at 1,500 rpm for 10 minutes using a Clay Adams ® TRIAC ® centrifuge (Becton Dickinson Company, Franklin Lakes, NJ). Serum was harvested from the samples and stored at -80° C until analysis.

Fecal sampling. Fecal samples were obtained opportunistically from a sheet of aluminum foil placed on the bottom of individual transport kennels during the circadian and the ACTH-challenge experiments. Samples were transferred into plastic 12 x 75 mm test tubes (VWR

International, West Chester, PA), and stored at – 80°C. The samples were dried using a Speed-Vac® Plus evaporator (SC110A; Savant Instruments, Holbrook, NY), crushed, and measured out in 0.02 to 0.03g aliquots. Fecal samples were extracted as described by Monfort *et al.* (1998). Dried samples were reconstituted in 650 µl of methanol (MeOH), then 100 µl of methanol extractant was transferred to a second set of test tubes which were dried using a manifold and reconstituted in 2 ml of buffer (ICN steroid diluent) yielding a 1:20 solution.

Circadian rhythms

Harlequin ducks were split into two groups of six animals each and sampled concurrently every four hours over a 24-hour period. At the time of the study (mid-April), the ambient daily light cycle was 14 hours light and 10 hours dark. Prior to sampling, the birds were herded into a holding pen and loaded into individual veterinary transport kennels. After sampling, birds were returned to the outdoor enclosures. An average of 3.9 ml (range: 3.5 to 4.1 ml) of whole blood was collected per bird during the 24 hr study period.

ACTH-challenge

Twelve harlequin ducks participated in an ACTH-challenge to characterize the stress response in this species. Birds in the treatment group (EXP, $N = 12$) received an intra-muscular injection of 0.5 ml (25 mg) of synthetic adrenocorticotropic hormone (ACTH; Cortrosyn®, Organon Inc., West Orange, NJ). Control birds (CTRL, $N = 5$) received the same volume of sterile saline. The ACTH study was performed in late April 2002. Five of the ducks had to be removed from the study due to low packed cell volume (PCV) values (less than 30 %). These birds participated in a second ACTH-challenge three weeks later. At the second ACTH-challenge, birds that had been in the treatment group were placed in the control group and vice versa. Hence,

five birds were subjected to both treatments. For "Time 0" samples, blood was drawn just prior to administration of ACTH or saline, and serial samples were drawn at 30, 60, 90, 120, 180, and 240 minutes after the injection. All samples contained 0.2 to 0.7 ml of blood.

Field samples

Serum samples were collected from wild harlequin ducks in Prince William Sound, Alaska (60° N; 148° W) during molting season in 1995, 1996, and 2002 (*see* Esler *et al.,* 2000).

Prince William Sound 1995 and 1996. Residual oil from the *Exxon Valdez* oil spill in 1989 was still present around Naked Island in 1995 and 1996, whereas Montague Island was not contaminated during the oil spill (Esler *et al.*, 2000; Trust *et al.*, 2000). Serum samples were obtained from harlequin ducks from areas around Naked Island and Montague Island during the first two weeks of September in 1995 and 1996. Kayaks were used to herd molting harlequin ducks into net pens. Birds were transferred to individual transport kennels where they spent between two and ten hours prior to radio-transmitter surgery. The time spent in kennels by each individual bird was not recorded (Esler *et al.*, 2000).

Prince William Sound 2002. Harlequin ducks were caught in Prince William Sound at the end of November/beginning of December 2002 as described above. A blood sample was obtained within minutes of capture (i.e., 3 to 17 min) before placing the birds in individual kennels. The time between capture and blood sampling was recorded for each bird. This was done in order to observe the relationship between time since capture and CORT concentrations. The birds were subsequently placed in kennels and a second sample was obtained three to six hours post capture. The second sample was obtained to investigate the influence of a continuous stressor (i.e., being held captive) on CORT concentrations several hours post capture.

Radioimmunoassay

A double antibody radioimmunoassay (ImmuChem™ Double Antibody Corticosterone ^{125}I RIA Kit, ICN Biomedicals, Inc., Costa Mesa, CA) was validated for use in harlequin ducks by performing a parallelism and an accuracy check for both serum and fecal samples. Parallelism was tested by comparing a 50 µl pool of harlequin duck serum or feces added to the kit standard calibrators with pure standard calibrators. The resulting standard curves were plotted after log-logit transformation (Rodbard, 1974). All serum samples were run at a dilution of 1:100 and all fecal samples at a dilution of 1:20 in the assay buffer provided in the kit. Non-specific binding and the sensitivity of the assay were recorded.

High pressure liquid chromatography (HPLC)

Immunoreactive glucocorticoid constituents in female harlequin duck serum and feces were established by using HPLC (Varian ProStar 210/215, Varian Inc., Walnut Creek, CA). Individual serum and fecal samples were selected at random and used to create 3 ml pools. The method used to analyze and collect fractions (1 to 80) of the serum and fecal pools by using HPLC has been described by Monfort *et al.* (1998). The immunoreactivity associated with CORT in the pools which co-elutes with radiolabeled (^{3}H) CORT on an 80 minute HPLC gradient (flow rate 1ml/minute) was evaluated.

Other variables

As part of the circadian rhythm experiment, the correlation between serum CORT concentrations and the following variables was also investigated: i) diet, ii) severity of bumblefoot, iii) packed cell volume (PCV), iv) heterophil:lymphocyte (H/L) ratio, v) time since capture, vi) restraint duration, vii) percent of blood volume collected, and viii) body mass. These

variables were only investigated (with the exception of H/L ratio) during the circadian rhythm study since the introduction of an exogenous stressor (e.g., synthetic ACTH) can obscure naturally occurring fluctuations in serum CORT. Samples from the ACTH study could be used to investigate the correlation between H/L ratio and CORT since it was the relationship between these two variables that was investigated rather than baseline values of the H/L ratio in harlequin ducks. Each variable is briefly described below.

Diet. A simultaneous study at the ASLC (Tuomi *et al.*, 2003) investigated the effects of a mixed diet with multi-vitamin supplements versus a single food-item diet without supplemental vitamins on the health of harlequin ducks. In this study, we compared serum CORT concentrations in animals undergoing the two diet regimes.

Bumblefoot severity. Bumblefoot is a disease syndrome described in several species of birds in captivity, manifesting as inflammatory and infectious foci in the feet. If left untreated, bumblefoot may progress to a lethal systemic illness (Smith-Ruiz, 1997). Causes of bumblefoot include injury to the foot, inactivity or inability to fly (Heidenrich, 1995), pressure, inadequate diet, depressed immune system, or inadequate substrate in the holding facility (Smith-Ruiz, 1997). We followed the classification scheme for bumblefoot severity in which the least severe case is assigned a "1" and the most severe a "7" (WildCare, San Rafael, CA). Average CORT concentrations were compared among birds showing various concentrations of bumblefoot and birds with no symptoms (i.e., "0").

Packed cell volume. PCV counts were performed periodically to ensure that the ducks did not become anemic and those with PCV values below 30 % were withdrawn from the study. The correlation between PCV and CORT concentration was analyzed.

Heterophil: lymphocyte ratio. In addition to CORT concentrations, the relative number of circulating leukocytes (i.e., the ratio of heterophils to lymphocytes) have been used to evaluate

stress in birds (Mitchell et al., 1992; Maxwell, 1993; Maxwell and Robertsson, 1995). An increased H/L ratio indicate a stress response, which typically occur much slower (in the order of hours and days) after exposure to a stressor as compared to the adrenal stress response (Vleck, 2001). The H/L ratio was determined by differential counts of 100 leukocytes from blood smears and by dividing the number of heterophils by the number of lymphocytes.

Time since capture, restraint duration. Time since capture was defined as the time between capture in the outdoor enclosures and the time of the blood draw. Restraint duration was defined as the time between the removal of the ducks from the individual kennels and the actual blood draw. Average time between capture and bleed was 16 min 18 sec (± 1 min 49 sec, range: 2 to 44 min). Mean bird handling time (i.e., restraint time) was 3 min 36 sec (± 20 sec, range: 1 to 12 min).

Percent of blood volume collected. Percent blood volume collected was defined as the total amount drawn during the 24 hr study divided by total blood volume (i.e.,10 % of body mass) multiplied by 100.

Statistical analyses

Statistical analyses were performed using SAS® package and Microsoft® SigmaStat. Values greater than two standard deviations from mean were omitted from the comparisons, and nonparametric tests were applied to account for small sample size. Repeated measures analysis was used to evaluate the variation of CORT concentrations in the circadian rhythm study. Rank correlations (i.e., Pearson and Spearman correlations) were used to examine the relationships between CORT concentrations and continuous variables such as body mass, PCV, percent blood volume collected, and the H/L ratio. An analysis of variance (ANOVA) was used to compare CORT concentrations and variables placed in time blocks or non-continuous variables, such as

time since capture, restraint time, blood collection site, and severity of bumblefoot. Differences in CORT concentrations between diet groups were compared using a Student's *t*-test. A Tukey Multiple Comparison Procedure was used to compare years and locations for the Prince William Sound field samples. A significance level of 0.05 was used for all statistical tests. Concentrations from fecal samples were pooled into two-hour time blocks due to the range in fecal deposition times and the uncertainty of the exact deposition time.

RESULTS

Assay validation

Serial dilutions (1:1 to 1:1024) of harlequin duck serum and fecal pools yielded curves parallel to the standard curve (Figs. 1-1a & b). A regression analysis on pools added to standard volume produced a slope of 1.022 (R^2 = 0.999), a mean recovery rate of 101.3 % for serum, and a slope of 0.647 (R^2 = 0.987) and a mean recovery rate of 80.6% for fecal samples (Fig. 1-2). The non-specific binding (NSB) and mean sensitivity (i.e., lower detection limit) for serum were 3.9% and 15.4 (SD: 4.2) ng/ml, respectively. Fecal sample NSB and mean sensitivity were 3.4 % and 14.5 (SD: 5.4) ng/g, respectively. Intra-assay and inter-assay variations were 3.0% and 9.0% for serum, and 3.0% and 6.1% for fecal samples, respectively.

Pooled female harlequin duck serum exhibited a peak of immunoreactivity which co-eluted with tritiated CORT (Fig. 1-3a). While similar elution profiles were observed between the fecal pool and added tritiated CORT, a large immunoreactive peak in the polar regions (i.e., lower fractions numbers) of the elution gradient was also apparent (Fig. 1-3b). This polar compound was not identified.

Circadian rhythms

Serum CORT concentration. Average CORT concentration in female harlequin ducks (N = 11) did not differ between sampling times (F = 1.079, P = 0.384) and no clear trend in mean CORT concentrations over a 24-hour period was found (Fig. 1-4). However, seven of the 11 females exhibited individual peak CORT concentrations in the morning hours (i.e., 05:00 and 09:00; Fig. 1-5).

Fecal CORT concentration. A total of 14 fecal samples were obtained during the 09:00, 13:00, 17:00, and 21:00 time blocks. Large individual variation in CORT concentrations was observed (Fig. 1-6). Mean fecal CORT concentrations for the first three time blocks ranged between 130 and 175 ng CORT/g feces while the mean for the 21:00 time block was about twice that (271 ng CORT/g, Fig. 1-6). Mean fecal CORT concentrations did not differ statistically among time blocks (H = 4.775, P = 0.311).

ACTH-challenge

Serum CORT concentration. Mean "Time 0" serum CORT concentrations did not differ (t = 0.266, P = 0.217) between the experimental and control group. Average serum CORT concentrations for the controls remained at "Time 0" concentrations (24.1 to 41.0 ng/ml) throughout the experiment (Fig. 1-7).

For the female harlequin ducks, the average peak serum CORT concentration occurred 90 minutes post ACTH administration (Fig. 1-7). Three females and one male exhibited peaks at 120 minutes (Fig. 1-7). Mean peak CORT concentration was approximately three times higher than baseline (146.2 ±12.7 ng/ml). The final value (i.e., 240 minute post-ACTH) did not differ significantly from baseline (t = -1.277, P = 0.217).

Fecal CORT concentration. Two to 13 fecal samples were obtained per two-hour time block from all birds combined. Large individual variations in CORT concentrations were observed (Fig. 1-8). Mean fecal baseline CORT concentrations in the ACTH study prior to injection of either ACTH or saline did not differ significantly (t = -0.621, P = 0.552). Mean fecal CORT concentrations in the ACTH group increased significantly (F = 6.223, P = 0.004) two to four hours post-injection. CORT concentrations decreased over the next four hours to not significantly below starting concentrations (Fig. 1-8). Mean CORT concentrations for birds in the CTRL group did not differ significantly among sampling times (F = 0.617, P =0.640, Fig. 1-8). CORT concentrations differed significantly between EXP and CTRL birds (F = 85.130, P = 0.012, Fig. 1-8).

Field samples

Prince William Sound 1995 and 1996. The average CORT concentration from samples obtained around Naked Island was higher than samples from Montague Island in both 1995 and 1996 (Fig. 1-10). However, the result was not statistically significant (F = 1.395, P = 0.241). A significant difference was found (q = 2.991, P = 0.038) between years for Montague Island but not for Naked Island (fig. 1-10).

Prince William Sound 2002. CORT concentrations increased with time since capture in samples obtained between two and 18 minutes of capture when CORT concentrations for each individual were plotted in a composite stress response curve (Fig. 1-11a). Samples obtained between three and six hours after capture exhibited high variability (over an order of magnitude) and no clear trend in CORT concentrations (Fig. 1-11b).

Other variables

Bumblefoot stages ("grades") were assessed between 0 and 3, on a scale of 0 to 7, with the 7th grade being the most severe.

There was no correlation between time since capture, restraint time, bumblefoot severity, diet, H/L ratio, percent of blood volume collected, or body mass and CORT concentrations (Table 1-1). There was a negative correlation between PCV values and CORT concentrations ($r = -0.461$, $P < 0.001$; Fig. 1-9).

DISCUSSION

Assay validation

The co-elution of tritiated CORT and the peak of glucocorticoid activity observed in both serum and fecal samples along with the tests for linearity and parallelism validated the RIA procedure described herein as a method of measuring concentrations of CORT in harlequin ducks (Figs. 1-1, 1-2, and 1-3). Along with routine quality control measures of non-specific binding, assay sensitivity and intra and inter assay variation, the CORT assay can reliably measure CORT concentrations in feces and blood serum. Furthermore, through the validation procedure CORT was confirmed as the main glucocorticoid metabolite in harlequin duck blood, whereas a large polar peak in the elution gradient may represent the main metabolite in harlequin duck feces.

Circadian rhythms

Serum CORT concentration. Circadian patterns in CORT production in birds have been shown to typically peak prior to dawn with lowest concentrations observed at late afternoon to dusk (Beuving and Vonder, 1978; Westerhof *et al.*, 1994; Nelson, 1997). The pattern observed in the harlequin ducks in this study was similar, with the lowest concentrations in mid to late

afternoon. However, no significant differences were measured. The time of the year when the study was conducted may have affected individual circadian patterns. Studies on harbor seals (*Phoca vitulina*) have shown the presence of a diurnal pattern in CORT concentrations during the summer, when sufficient sunlight was available to act as an efficient environmental cue or *zeitgeber*, and an absence of a pattern during the winter months, when insufficient sunlight failed to produce a clear cyclical pattern (Daan and Gwinner, 1989; Oki and Atkinson, 2004). The harlequin duck females exhibited CORT peaks at different times, with most individuals peaking in the morning. It may be that there are individual differences in sensitivity to sunlight as the *zeitgeber,* or that spring months may be a period of adjustment, resulting in variation in the daily pattern of hormone production among individuals similar to that observed in April. Some individuals may have more rapidly adjusted to the upcoming long arctic summer in terms of regulating hormone production. It is therefore recommended to repeat this study in mid-summer and winter months with corresponding high and low sunlight concentrations.

Fecal CORT concentration. No clear trend in fecal CORT concentrations over time was observed, probably because of similar factors described above for serum CORT concentrations, and maybe because of a small data set. However, the concentrations obtained from the circadian rhythm study as well as "Time 0" concentrations for ducks in the treatment group and all time values for control birds in the ACTH challenge suggest that baseline CORT concentrations in female harlequin ducks are between 100 and 200 ng/g feces.

ACTH-challenge

Serum CORT concentration. The observed three-fold increase of basal CORT concentrations following the introduction of exogenous ACTH is similar to results from American black ducks (*Anas rubripes*; Spelman *et al.,* 1995). Other avian ACTH studies have

found a five-fold increase in Florida sandhill cranes (*Grus canadensis pratensis*; Ludders *et al.*, 1998) to a nine-fold increase in domestic ducks (*Anas platyrhynchos*; Harvey *et al.*, 1980). The time-lag between the stressor and the peak concentration in our study was similar to the lag-time found in American black ducks (90 vs 120 minutes), but differs from that found in some other avian species: 270 minutes for Moluccan Cockatoos (*Cacatua moluccensis*; Walsh *et al.,* 1985) and 60-90 minutes for bald eagles (*Haliaeetus leucocephalus*; Zenoble *et al.*, 1985). The sharp increase and the subsequent gradual tapering off of CORT concentration observed in this study contrasts with the post-ACTH CORT profile for wild breeding male Gambell's white-crowned sparrow (*Zonotrichia leucophrys gambelii*) where a steep increase in CORT during the initial 10 minutes was followed by a 2 hour plateau at elevated concentrations (Astheimer *et al.,*1994).

The absence of increased serum CORT in the harlequin duck control group indicates that these birds were not stressed by handling or blood-draws. The birds participating in this experiment were handled on a weekly basis for six months prior to this study and, therefore, likely habituated to capture and handling. El Halawani *et al.* (1973) observed habituation in the CORT response to other stressors in turkeys over several weeks.

Fecal CORT concentration. Fecal CORT concentrations in female harlequin ducks peaked between two and four hours after ACTH injection, similar to Florida sandhill cranes, which were reported to peak around two to three hours after ACTH administration with concentrations slowly tapering off over the next few hours (Ludders *et al.,* 2001). Goymann *et al.* (2002) found fecal CORT concentrations in European stonechats (*Saxicola torquata rubicola*) to peak at around 1 hr 20 min post ACTH whereas the fecal CORT concentration in northern spotted owls (*Strix occidentalis caurina*) increased steeply about two hours after ACTH administration, with the peak concentration at 12 hours post injection (Wasser *et al.*, 1997). The observed four to five fold increase in fecal CORT concentrations from baseline to peak in the birds in the current

study is comparable to those observed in other avian ACTH studies (Wasser *et al.*, 1997; Ludders *et al.,* 2001; Goymann *et al.,* 2002). The results obtained in the present study suggest that fecal samples can be used to noninvasively assess stress hormones in harlequin ducks.

Field samples

Prince William Sound 1995 and 1996. The highest average CORT concentrations in 1995 and 1996 were found in samples from harlequin ducks at Naked Island, where residual oil from the *Exxon Valdez* was still present (Esler *et al.*, 2000, Trust *et al.*, 2000). Although not statistically significant, this finding may be biologically significant and might be an indicator of the birds' adrenal response to the oil still in the area. Correlations have been found between external petroleum fouling and oil-ingestion and elevated CORT concentrations in other avian and non-avian species (mallards, *Anas platyrhynchos*, Holmes *et al.*, 1979; Magellanic penguins, *Sphenicus magellanicus,* Fowler *et al.*, 1995; marine iguanas, *Amblyrhynchus cristatus*, Romero and Wikelski 2002). The inter-annual variation in CORT concentrations observed in harlequin ducks molting around Montague Island has been found in studies on other bird species (semipalmated sandpipers, *Calidris pusilla*, Mizrahi *et al.*, 2001).

Prince William Sound 2002. The linear relationship between CORT concentrations and time since introduction of a stressor (i.e., capture) observed in harlequin ducks in PWS 2002 is characteristic of the adrenal stress response and has been shown for many avian species (Wingfield *et al.*, 1982; Astheimer *et al.*, 1995; Pravosudov *et al.*, 2001; Cockrem and Silverin, 2002a). However, the magnitude of the stress response was much greater in the present study as compared to the study by Perfito *et al.*, (2002). At 15 min post-capture, Prince William Sound birds had increased in blood CORT concentrations from around 30 ng/ml to around 100ng/ml whereas the corresponding concentrations in the study by Perfito *et al.*, (2000) in Washington

state were 30 ng/ml to around 40 ng/ml. The highest CORT concentrations reported by Perfito *et al.*, (2002) for molting harlequin ducks were around 45 ng/ml at 30 min post capture, after which the concentrations decreased. In contrast, harlequin ducks in the present study, which had spent between three and six hours in pet kennels, exhibited CORT concentrations that ranged from around 20 to 200ng/ml, most being between 50 and 150 ng/ml. Whereas the specific reasons for the much higher CORT concentrations observed in the present study are unknown, they may include differences in capture method (Perfito et al., 2002), geographical location (Silverin et al., 1997; Silverin and Wingfield, 1998), and RIA methods. Individual differences in the shape and magnitude of the stress response curve may account for some of the high variability observed in samples obtained 3 to 6 hours post capture. Individuals may also have been more or less susceptible to the stressors they were exposed to in this study (i.e., capture and confinement for several hours). Although no trend in CORT concentrations over time was observed, it is likely that the time spent in the kennels influenced individual CORT concentrations.

Other variables

The absence of a relationship between either capture and bleed or handling time and CORT concentrations is likely due to habituation to handling in these birds (see above). Although no relationship between time since capture time and CORT concentrations was observed, the large range of times between capture and bleed may have affected CORT concentrations in this study.

The lack of a relationship between bumblefoot severity and CORT concentration may have been because the degree bumblefoot was not severe in any of the birds in this study and therefore did not adversely affect their health or baseline CORT concentrations.

The strong negative relationship between PCV and CORT concentration observed in the present study suggests either i) that CORT concentrations increase as the individual becomes progressively more anemic or ii) that PCV decreases as CORT concentration increases.

The lack of correlation between CORT concentrations and H/L ratio can be explained by the difference in response time, the adrenal stress response occurring within minutes of exposure to a stressor, and the leukocyte response time occurring within hours to days (Vleck, 2001).

The absence of a relationship between percent of blood volume collected and CORT concentrations is probably due to the relatively low volumes collected. No physiological stress reaction due to blood-loss was expected at these low volumes of blood collected. For birds, it is considered safe to collect a blood volume of one percent of body weight (Dein, 1984).

In conclusion, the double-antibody RIA used in this study was validated to measure CORT concentrations in harlequin duck serum and fecal samples. Circadian patterns of serum CORT showed large variation among individuals during the month of April in Alaska, and it is therefore recommended that future studies investigating circadian rhythms in sea ducks be conducted during both mid-summer and winter months. Harlequin ducks showed a substantial increase in both serum and fecal CORT concentrations in response to an ACTH stimulation. The results indicate that fecal samples can be used to non-invasively measure corticosterone in harlequin ducks.Future studies investigating the effects of short term captivity (i.e., hours) on glucocorticoid concentrations in sea ducks should consider using fecal samples as a non-invasive complement to repeated serum sampling.

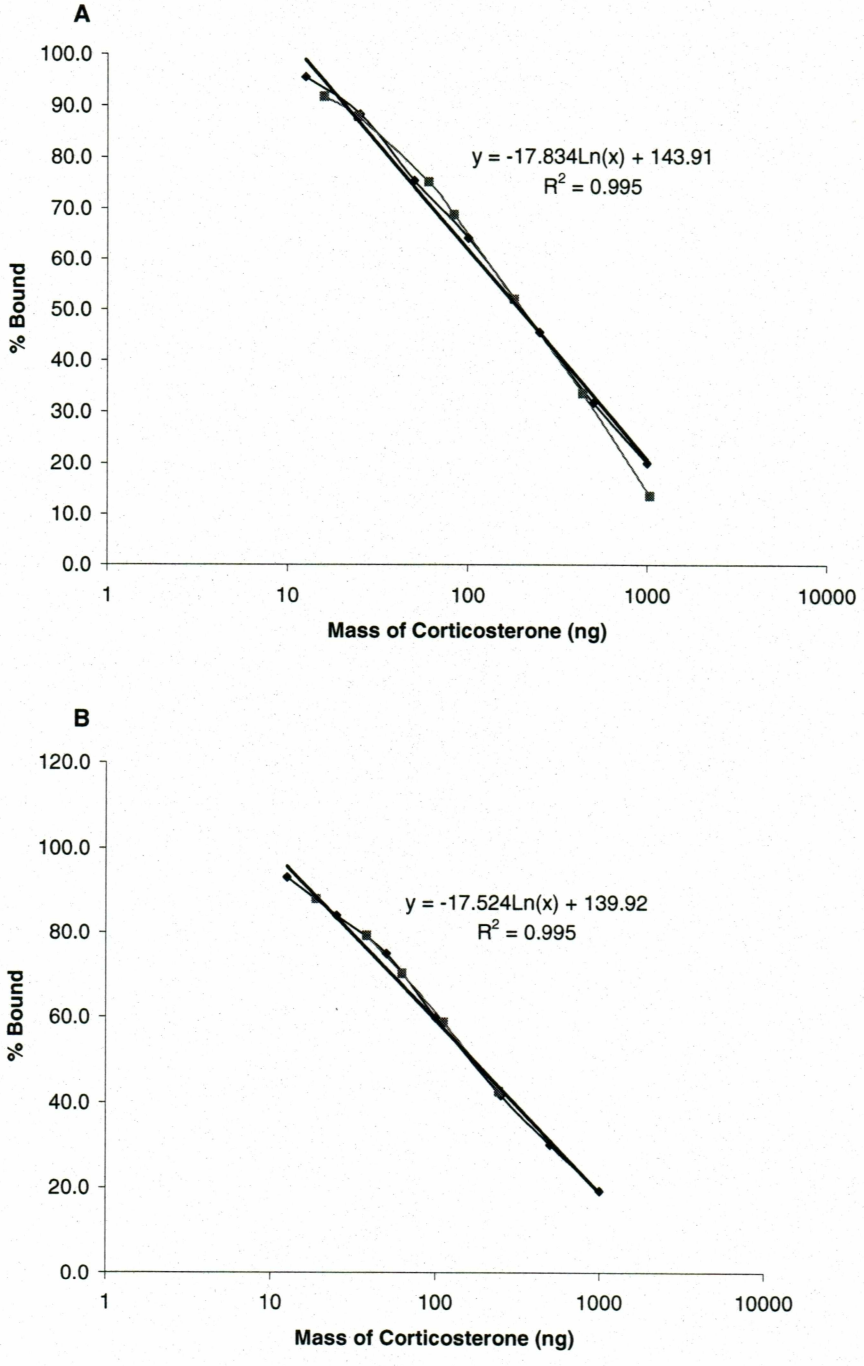

FIG. 1-1. Parallelism of corticosterone mass in harlequin duck serum (a) and feces (b) with assay standard curve. Curves of percent binding of ^{125}I corticosterone versus serially diluted pooled serum and feces were parallel to the assay standard curve.

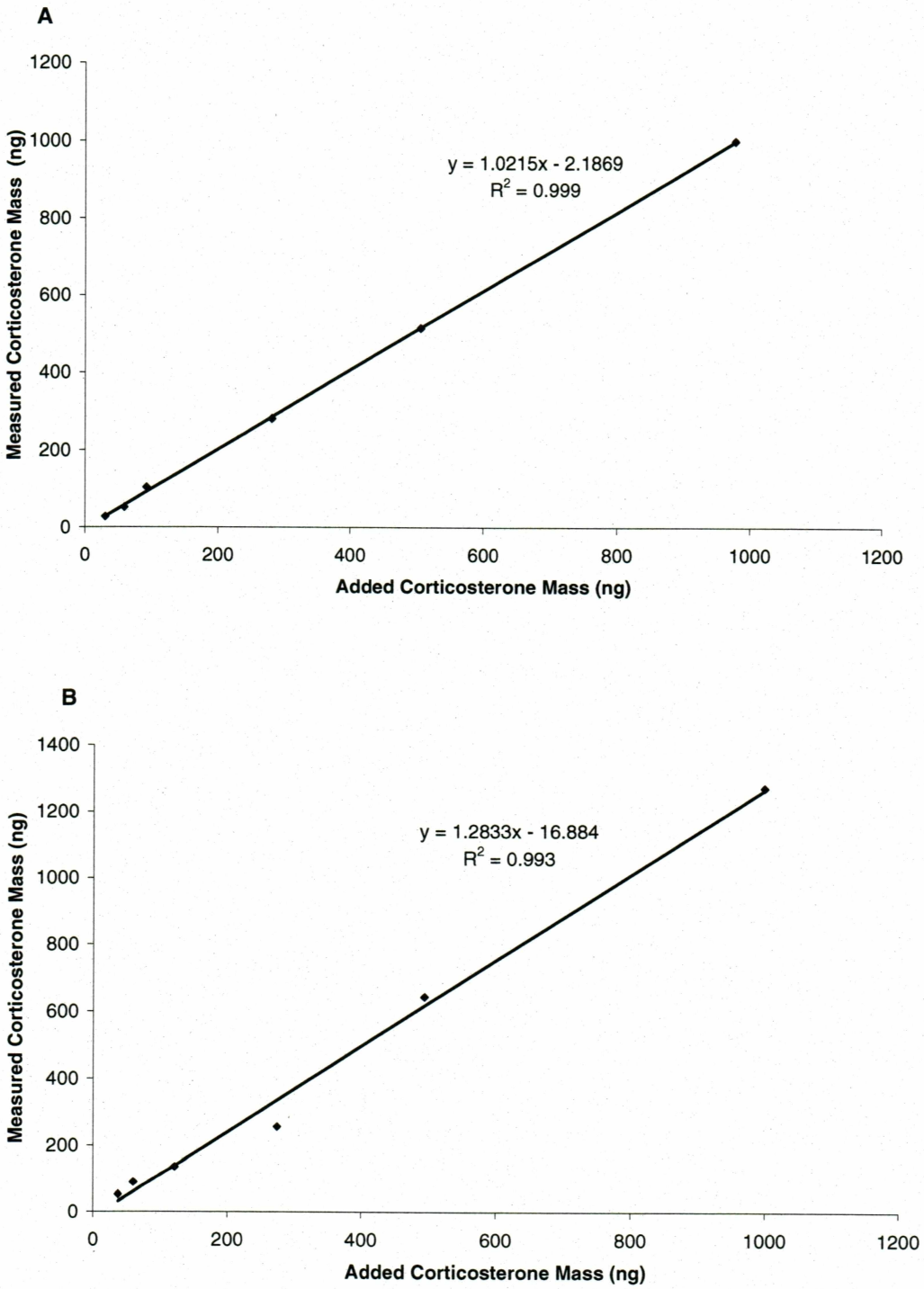

FIG 1-2. Accuracy check for female harlequin duck corticosterone radioimmunoassay. Regression analysis of pooled (a) serum and (b) feces added to standard curve.

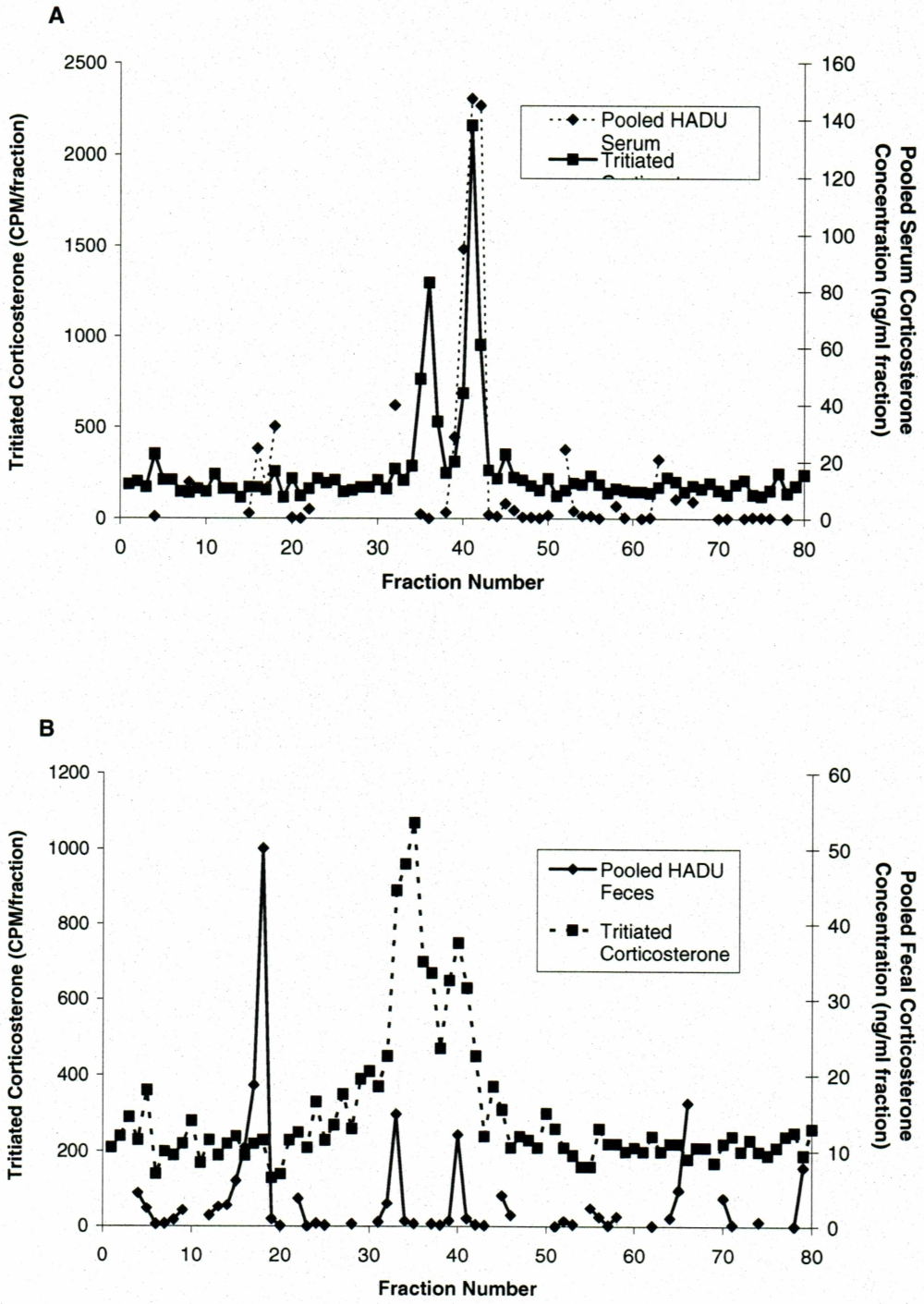

FIG. 1-3. Immunoreactive corticosterone HPLC profiles of harlequin duck (a) serum and (b) feces. ^{3}H corticosterone was added as a reference.

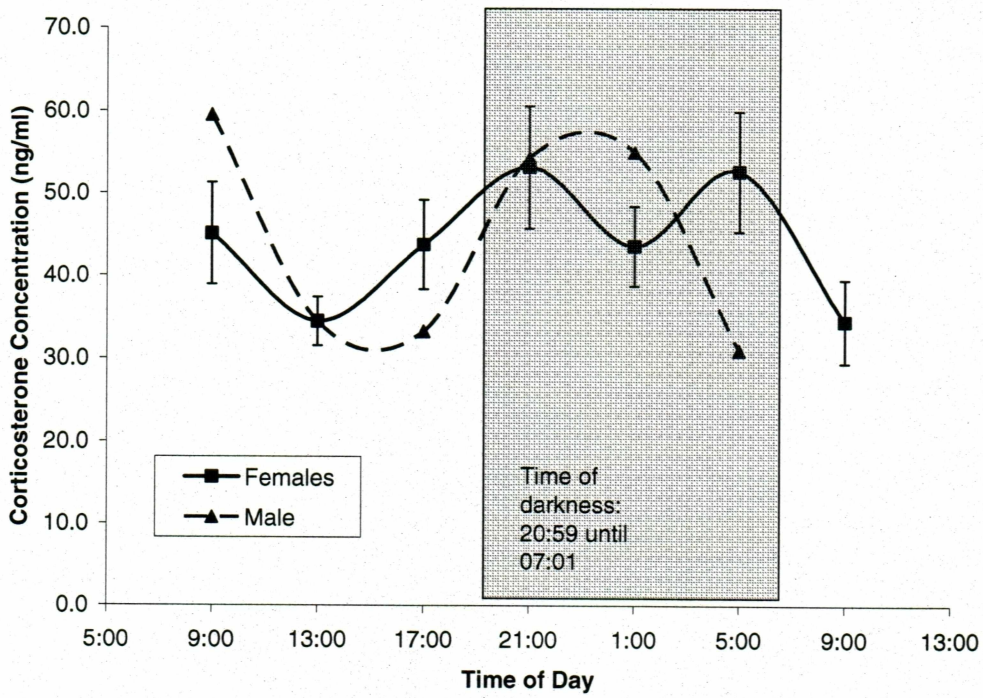

FIG. 1-4. Variation in mean (±SE) serum corticosterone concentrations (ng/ml serum) over a 24-hour period for female ($N = 11$, solid line) and male ($N = 1$, dashed line) harlequin ducks. The study was performed in Seward, Alaska (60° 69' N; 149° 26' W) under ambient light conditions in mid-April. The average serum CORT concentration did not differ significantly between sampling times ($F = 1.079$, $P = 0.384$).

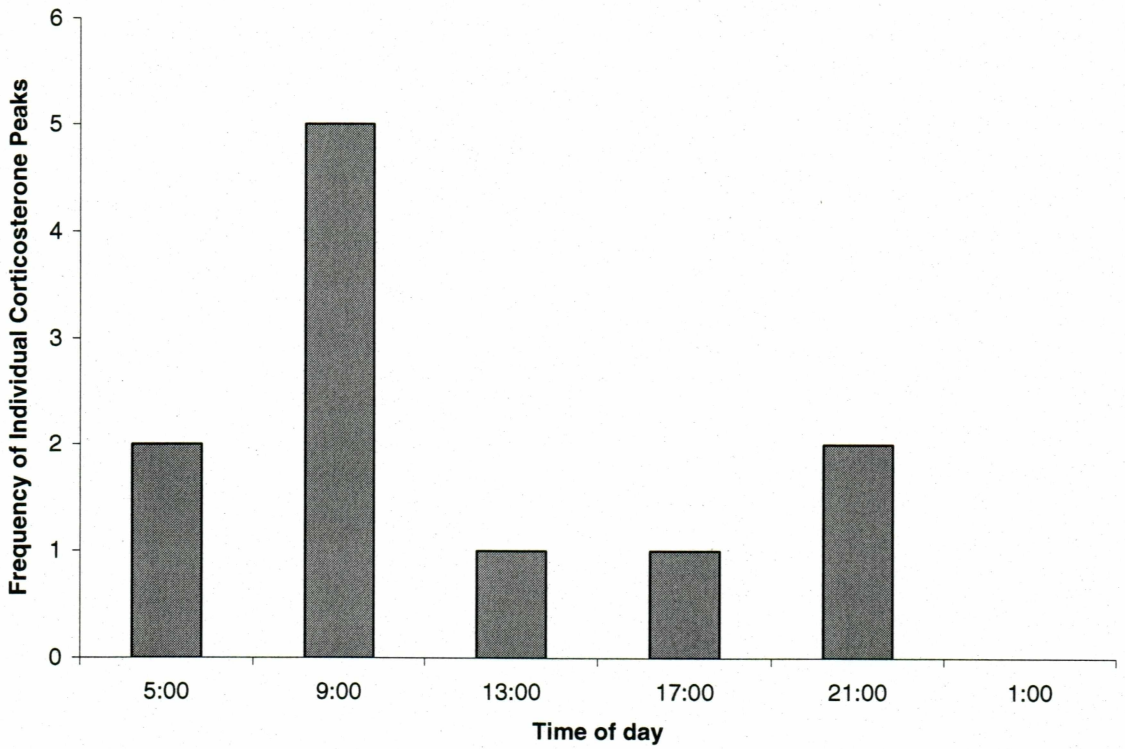

FIG. 1-5. Distribution of individual corticosterone peaks in serum of 11 female harlequin ducks during a 24-hour period. The study was conducted in Seward, Alaska (60° 69' N; 149° 26' W) under ambient light conditions in mid-April.

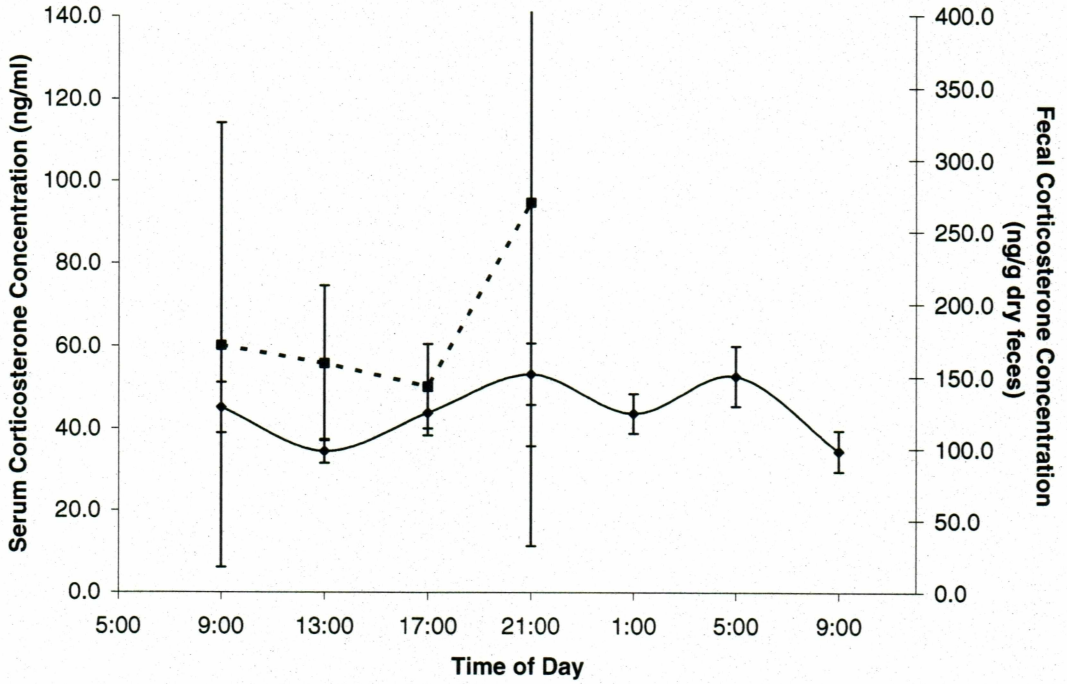

FIG. 1-6. Mean (± SE) fecal (dashed line) and serum (solid line) corticosterone concentrations in female harlequin ducks over a 24-hour period. Mean CORT concentrations did not differ significantly between sampling times (serum: $F = 1.079$, $P = 0.384$; fecal samples: $H = 2.886$, $P = 0.410$).

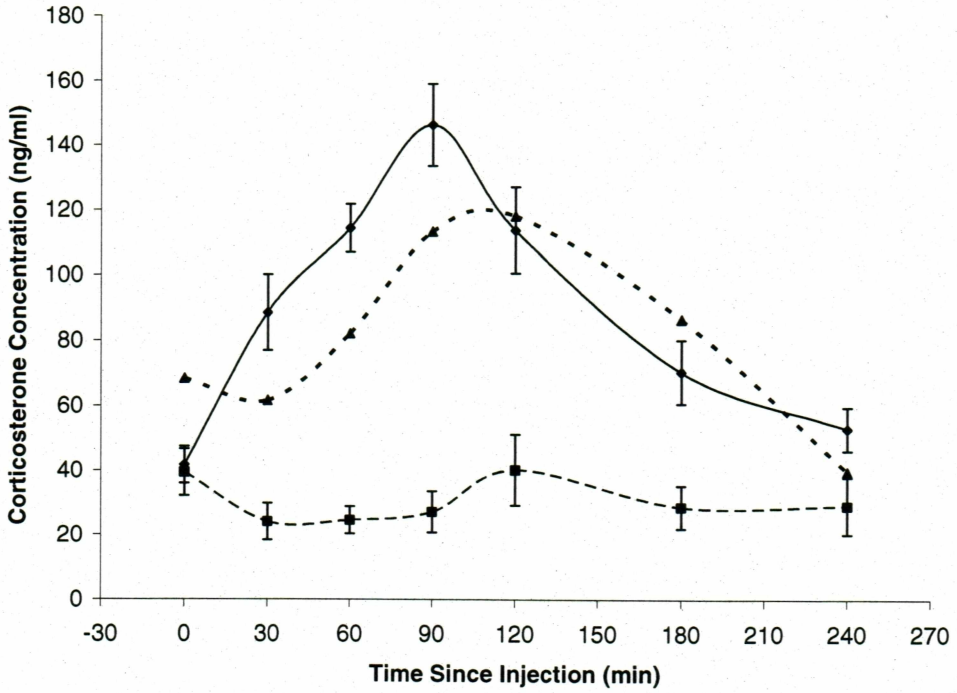

FIG. 1-7. Serum corticosterone profiles in harlequin ducks (16 females, one male) after injection of synthetic ACTH (females: solid line, male: dotted line) or saline (dashed line). ACTH/saline injection at 0:00, first sample collected prior to injection.

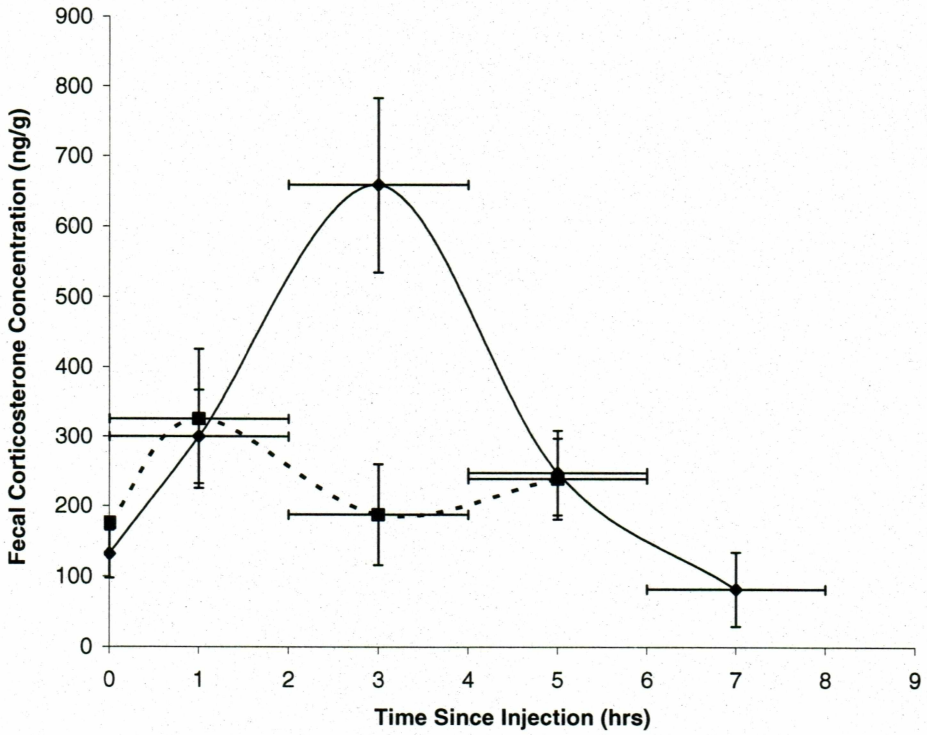

FIG. 1-8. Fecal corticosterone concentrations in captive female harlequin ducks after injection of synthetic ACTH (solid line) and saline (dashed line). Fecal samples were pooled into two-hour time blocks due to the uncertainty of exact time of deposition within that time period (horizontal bars). The 2 to 4 hour time block differed significantly from the other time blocks ($F = 6.223$, $P = 0.004$).

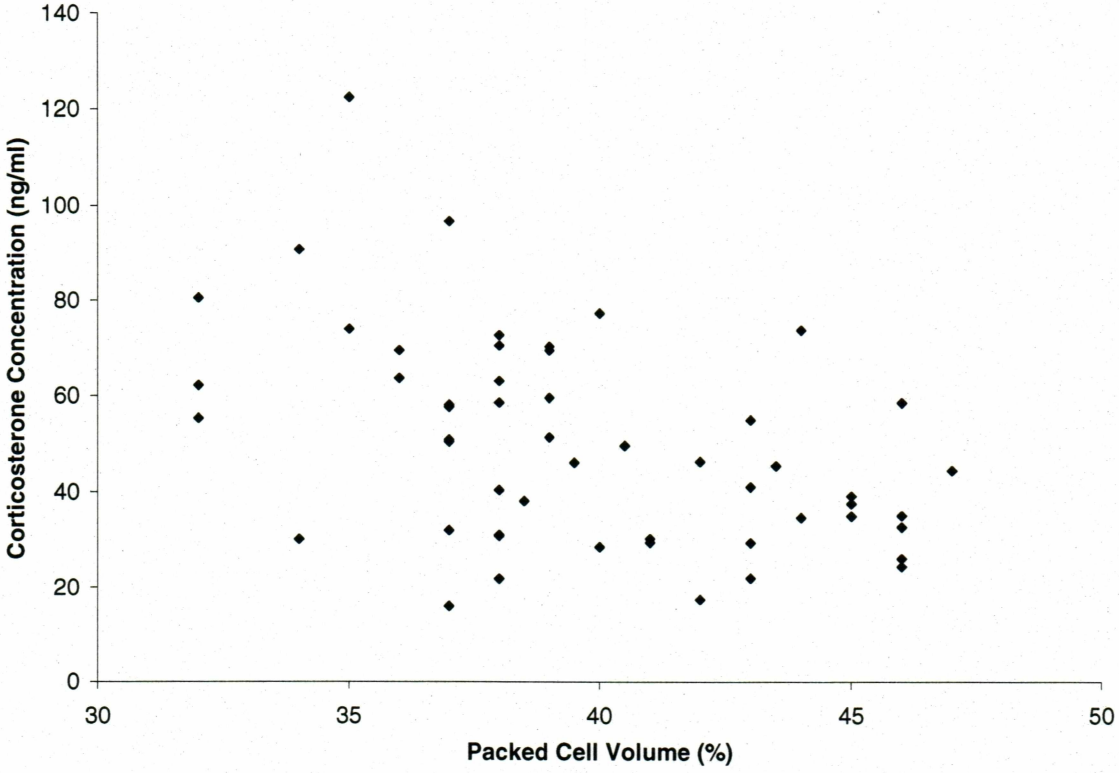

FIG. 1-9. Correlation between corticosterone concentration and packed cell volume (PCV) in female harlequin ducks ($r = -0.461$, $P < 0.001$).

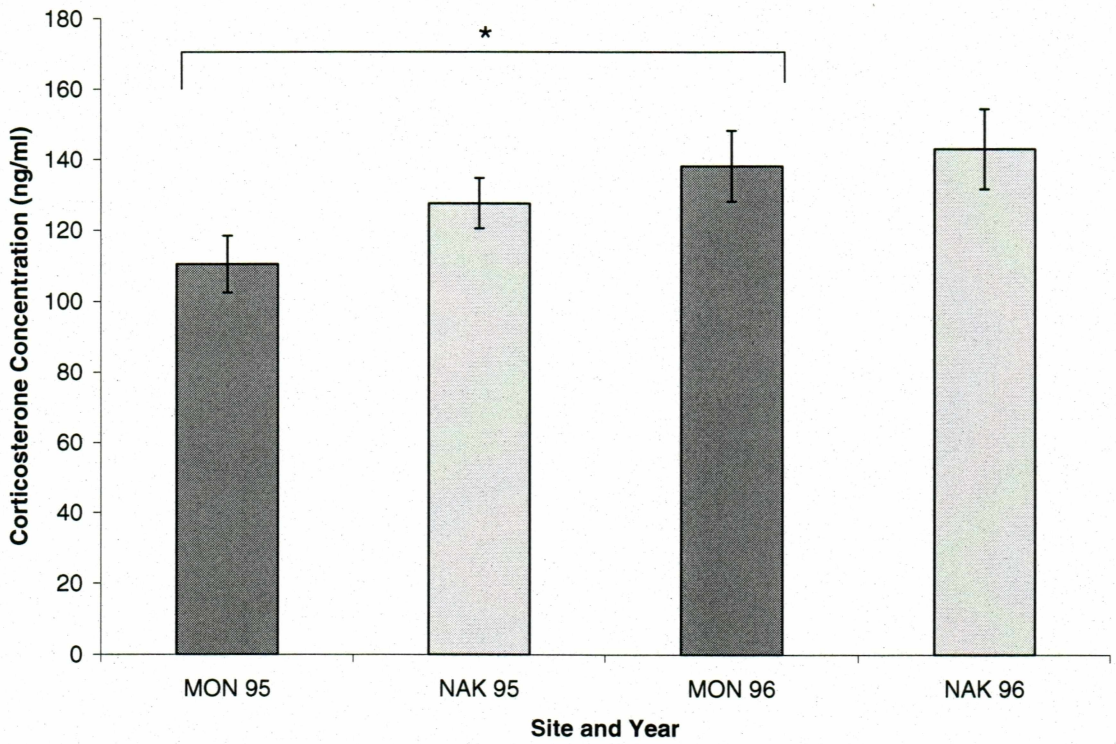

FIG. 1-10. Mean (± SE) circulating corticosterone concentrations in two populations of harlequin ducks in Prince William Sound, Alaska (60° N; 148° W) in 1995 and 1996. The mean represents CORT concentrations in samples obtained between two and eight hours after capture. Residual oil from the *Exxon Valdez* oil spill in 1989 was present in the area around Naked Island (NAK) but not around Montague Island (MON) during 1995 and 1996. The only significant difference found was between 1995 and 1996 for Montague Island ($*q = 2.991$, $P = 0.038$).

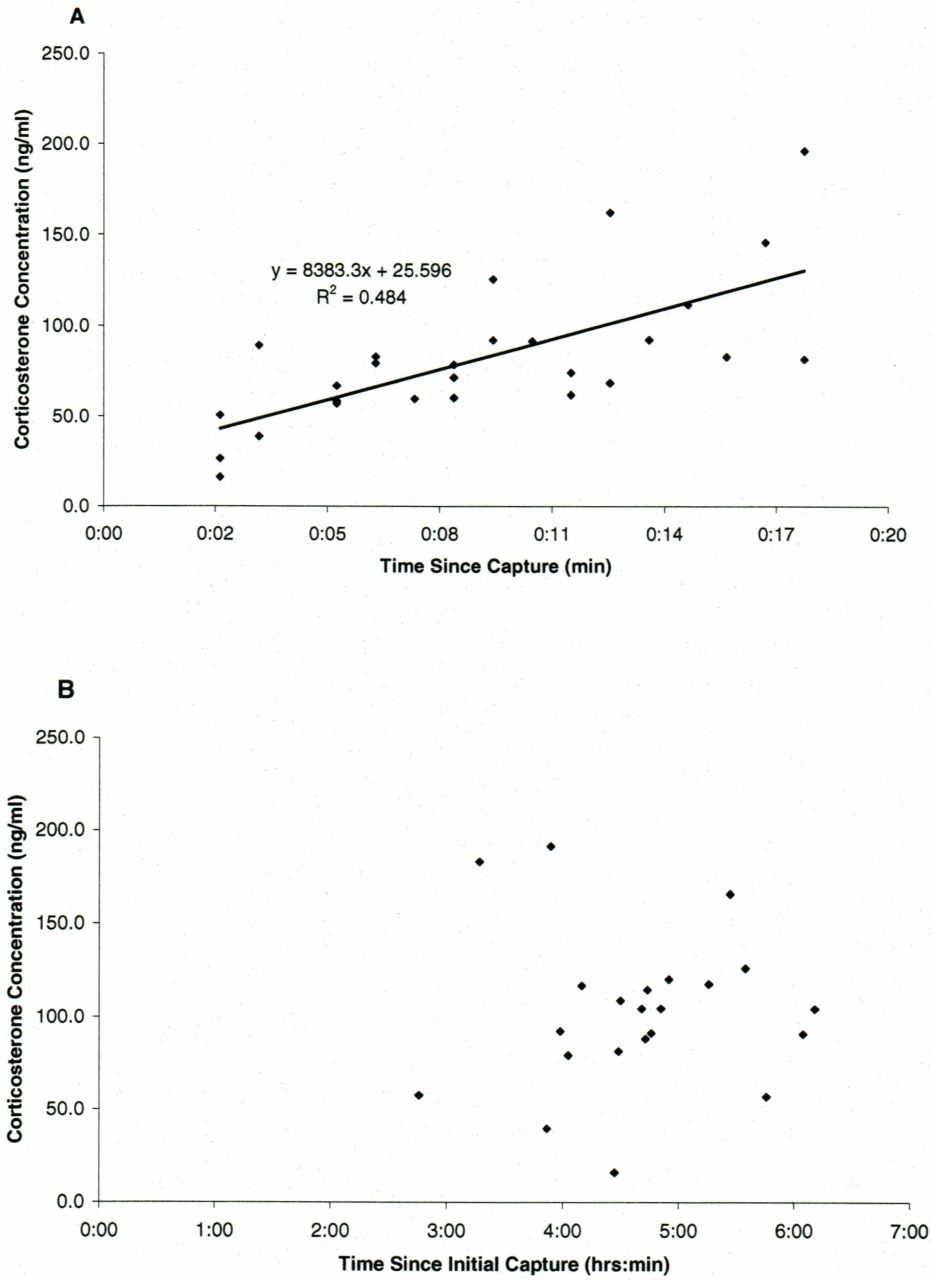

FIG. 1-11. Corticosterone response to (a) capture (y = 838.3x + 25.596, $r^2 = 0.485$, $P < 0.01$) and to (b) short term captivity (two to seven hrs post capture; y = 18.562x + 98.825, $r^2 = 0.0002$, $P = 0.943$), in wild harlequin ducks in Prince William Sound, Alaska (60° N; 148° W). Each data point represents a different individual.

TABLE 1-1. Corticosterone concentrations versus other variables in female harlequin ducks

Pearson Correlation		
Variable	*r*	*P*
Time since capture	0.036	0.797
Restraint duration	0.123	0.384
Percent of blood volume collected*	0.459	0.155
Body mass	-0.467	0.147
One-Way Repeated Measure ANOVA		
Variable	*F*	*P*
Bumblefoot Severity	0.772	0.549
Mann-Whitney Rank Sum Test		
Variable	*t*	*P*
Diet**	-1.374	0.174

Note: * Percent of blood volume collected refers to the total amount of blood withdrawn over a 24 hour period divided by blood volume (i.e. 10 % of body mass) multiplied by 100. ** One group of harlequin ducks was fed a restricted diet without vitamin supplements whereas one group was fed a more complete diet with additional vitamin supplements.

Chapter 2. Characterizing corticosterone baseline and response in eiders, genera *Somateria* and *Polysticta*[2]

INTRODUCTION

Several populations of sea ducks (tribe *Mergini*), have declined during the past two to three decades (Stehn *et al.,* 1993; Henny *et al.*, 1995; Suydam, 2000; Sea Duck Joint Venture Management Board, 2001). These population declines are particularly evident among the four species of eider ducks, a group of sea ducks inhabiting high latitudes in the northern hemisphere (Bellrose, 1980; Gooders and Boyer, 1986). Local populations of common eiders (*Somateria mollissima*) have declined drastically during the past two to three decades (Robertson and Gilchrist, 1998; Sea Duck Joint Venture Management Board, 2001). Declines of 53% and 56% in Pacific common eider (*S. mollissima v-nigra*) and king eider (*Somateria spectabilis*) numbers, respectively, migrating past Point Barrow, Alaska between the years 1976 and 1996 were reported by Suydam *et al.* (2000). A decline of >95% of the breeding population of spectacled eiders (*Somateria fischeri*) occurred in the Yukon-Kuskokwim (Y-K) Delta between the early 1970's and 1992 (Stehn *et al.*, 1993). The spectacled eider was listed as "threatened" under The Endangered Species Act in 1993 (Federal Register, 1993). Similarly, the Alaskan breeding population of Steller's eiders (*Polysticta stelleri*) was listed as "threatened" under The Endangered Species Act in 1997 (Federal Register, 1997). The designation was prompted by the apparent disappearance of Steller's eiders as a breeding species in Y-K Delta (Kertell, 1991). Since then, a few nests have been discovered in the area, however, the Steller's eider is currently considered a rare breeding species in Alaska (Flint and Herzog, 1999).

[2]Prepared for submission in *Condor* as Nilsson, P., T. Hollmén, and S. Atkinson. Characterizing corticosterone baseline and response in eiders, genera *Somateria* and *Polysticta.*

Potential threats and causes for these declines include lead poisoning from accidental ingestion of spent lead shots (Franson *et al.*, 1995; Flint *et al.,* 1997; Grand *et al.,* 1998), exposure to elevated concentrations of other trace elements such as copper and selenium (Stout *et al.*, 2002; Savinov *et al.*, 2003), petroleum fouling and ingestion following oil spills (Flint *et al.*, 1999), viral disease (Hollmén *et al.*, 2000), hunting (Circumpolar Seabird Working Group, 1997; Sea Duck Joint Venture Management Board, 2001), and starvation due to depletion of shellfish populations by commercial fisheries (Camphuysen *et al.*, 2002).

Many studies have shown how natural and anthropogenic threats or stressors influence concentrations of stress hormones in birds (Holmes *et al.*, 1979, Kitaysky, 2001, Ruiz *et al.*, 2002, Smith *et al.*, 1994). Natural threats which have been shown to increase stress hormone concentrations in birds include the presence of a predator (Cockrem and Silverin, 2002b), inclement weather (Smith *et al.*, 1994), and insufficient food supply (Kitaysky *et al.*, 1999), whereas anthropogenic stressors include oil ingestion (Holmes *et al.*, 1979) and human encroachment (Ruiz *et al.*, 2002).

Stress hormones are produced in the hypothalamo-pituitary-adrenocortical (HPA) axis and subsequently released into the blood stream (Norris, 1997; Norman and Litwack, 1998). Stress hormones fluctuate on a daily, seasonal, and annual scale (Breuner *et al.*, 1999; Mizrahi *et al.*, 2001; O'Reilly and Wingfield, 2003). The primary stress hormone in birds is corticosterone, CORT (Wingfield *et al.*, 1994a; Silverin, 1997; Wingfield *et al.*, 1998), which influences a host of physiological and behavioral events such as gluconeogenesis (McEwan and Stellar, 1993), hyperphagia (Koch *et al.*, 2001), and dispersal behavior (Silverin, 1997). The rapid increase of CORT concentrations (i.e., the stress response) is a physiological short-term survival mechanism, where the organisms' physiology and behavior are directed towards survival and all other non-essential activities are suspended. The increase in CORT related to the acute stress response is

thus beneficial to the individual, whereas chronically elevated CORT concentrations can have seriously deleterious effects such as suppression of the immune system (Wingfield *et al.*, 1998). Chronically elevated CORT concentrations in many individuals of a population can potentially indicate a struggling population and could be used as a biomarker to identify populations or areas where more focused research efforts should be implemented (Romero and Wikelski, 2002). Determining baseline CORT concentrations for a species and identifying temporal (e.g., diurnal and seasonal) patterns of CORT production, as well as differences among sub-populations, is necessary in order to determine which concentrations are "normal" and which concentrations may be indicative of a population under chronic stress.

The two main objectives in the present study were: i) to validate a radioimmunoassay (RIA) procedure for use in Steller's, spectacled, common, and king eiders, and ii) to characterize serum CORT concentrations in the four species of eider ducks with focus on Steller's and spectacled eiders. For these two species, the study sought to investigate differences in baseline CORT serum concentrations between: i) captive and wild ducks, ii) sexes, iii) nesting and molting seasons, and iv) among years. For common eiders, the aims were to examine differences in CORT concentrations in nesting females between: i) breeding sites, ii) years, and iii) to compare these to CORT concentrations in nesting female spectacled eiders. In addition, the study aimed at establishing CORT concentrations in male and female king eiders undergoing surgery for radio-transmitter implantation.

METHODS

Steller's eider

Field samples. Samples were obtained from molting Steller's eiders (STEI) during their flight feather molt at Izembek Lagoon (55° 16' N, 162° 54' W) on the north coast of the Alaska

Peninsula, Alaska. Once a large flock of STEI was located, aluminum skiffs and inflatable boats equipped with outboard engines were utilized to herd the flightless eiders into funnel-traps made of netting and metal poles. The technique is described in more detail in Flint *et al.* (2000). Approximately 1500 STEI were caught in one drive in 2001 and approximately 700 to 800 eiders were caught in each of three drives performed on three consecutive days in 2003.

A 22 gauge needle and 5 ml syringe were used to withdraw 4 ml of blood from the jugular vein. The blood was centrifuged in a Clay Adams ® TRIAC ® centrifuge (Becton Dickinson Company, Franklin Lakes, NJ) at 1500 rpm for 10 minutes within four to five hours of the blood draw. The serum was harvested and frozen in a dry shipper with liquid nitrogen (ca. -150° C).

Blood samples from three males and three females were collected near Barrow on the North Slope of Alaska (71° 17' N, 156° 47' W) during the 2001 breeding season.

Captive samples. Samples were collected from a captive flock of sexually mature STEI (seven males, five females) residing at the Alaska SeaLife Center in Seward, Alaska (60° 69' N, 149° 26' W).

Blood samples (3.5 ml) were collected by venipuncture of the right jugular vein during the breeding and molting seasons (July and October, respectively). The blood was centrifuged and the serum subsequently frozen and stored at -80°C until assayed for CORT concentrations. Average time between capture and bleed was 42± 4minutes (range 10 to 78 minutes).

Spectacled eider and Pacific common eider

Field samples. Samples were obtained from female spectacled eiders (SPEI) breeding in the Y-K Delta, Alaska (61° 20' N, 165° 35' W) during 2002 and 2003. Samples also were collected from female Pacific common eiders (COEI) breeding in the vicinity of Prudhoe Bay on

the North Slope of Alaska (70° 20' N, 148° 21' W; 2001 and 2003) and in the Y-K Delta (2003). Breeding COEI females from the North Slope were captured on the nest using bow-nets as described in Flint and Grand (1997). Breeding female SPEI and female COEI from the Y-K Delta were caught by mist nets dragged over the nest or by bow-nets. Blood (5 ml) was withdrawn via the right jugular vein within 10 minutes of capture for all years and sites except for COEI females on the North Slope in 2001 when the blood was collected via the brachial vein within 3 minutes of capture. The blood was centrifuged at 1500 rpm for 10 minutes within four to five hours of the blood draw, frozen in a dry shipper in the field, and kept frozen at -80 until analysis.

Captive samples. Samples from captive-bred 2nd year SPEI (six males, six females) residing at the ASLC were obtained during breeding and molting seasons of 2003. Blood samples (4 – 5 ml) were collected and stored as described above for captive STEI. Average time between capture and bleed was 46±7 min (range 13 to 89 minutes).

King eider

King eiders (KIEI) of both sexes were caught in mist nests in July 2003 in the vicinity of the Kagloryuak River in the Canadian arctic (70°16' N, 109° 59 W). After capture the eiders were placed in individual transport kennels and then transported via sled or backpack to a surgery tent to have radio-transmitters implanted. Blood samples were obtained either during anesthesia (N = 2), post-surgery ("Post-op"; N = 5) or when the birds had recovered (N = 3).

Radioimmunoassay (RIA)

A double antibody RIA (ImmuChem™ Double Antibody ^{125}I Corticosterone RIA Kit, ICN Biomedicals, Inc., Costa Mesa, CA) was validated for use in Steller's, spectacled, common and king eiders. Parallelism was tested by comparing a 50 µl pool of serum from each species of

eider added to the kit standard calibrators to the standard curve without serum (Rodbard, 1974). Accuracy checks were performed by calculating the mean recovery rate and plotting observed versus expected CORT concentrations to confirm that the slope and r^2 were not significantly different, indicating validity of the assay. In addition, routine quality control measures included the analysis of non-specific binding, sensitivity, intra and interassay variation.

Data analysis

Sigmastat® was used for statistical analyses. Outliers (values greater than two standard deviations from the mean) were excluded from further analyses. A parametric ANOVA was used on normally distributed data and a non-parametric ANOVA (Kruskal-Wallis) was used on data sets with small sample sizes and non-normally distributed data. An alpha level of 0.05 was used to indicate statistical significance. The concentrations for the 2001 Izembek molting STEI were placed in 15 minute time blocks and the means were analyzed using ANOVA. The start of the boat based-drive and the drive into the corral will be referred to in terms of Time Since Start of Drive (TSSD) and Time Since Capture (TSC), respectively. Because of the timing of sample collection, samples collected from molting STEI in Izembek in 2001 and 2003 were more appropriate for investigating stress responses over time from a stressor instead of investigation of baseline concentrations. Hence, these concentrations were not compared to wild breeding or captive STEI where samples were collected shortly after capture and as such more accurately reflect baseline concentrations. KIEI of both sexes were divided into three groups based on the timing of the blood draw in relation to the surgery (i.e., "anesthesia", "post-op", and "recovered"), and the means were compared using ANOVA.

RESULTS

Radioimmunoassay (RIA)

Parallelism. The slopes of the eider serum pool added to the standards and the standard curve differed by less than 5 % and were considered functionally identical (Fig. 2-1).

Accuracy check. The slopes produced by regression analysis on pool added to standard volume and the mean recovery rate indicated that the increased mass of CORT added resulted in a linear increase in the amount measured indicating that the assay was valid for all eider species (Table 2-1). Serial dilutions of male and female STEI serum and female COEI and SPEI serum showed optimal binding (approximately 50 %) at around a 1:50 dilution in assay buffer provided in the kit (ICN® steroid diluent). Male and female KIEI showed optimal binding at a 1:100 dilution in the assay buffer. These were the dilutions subsequently used for each species.

The mean non-specific binding was 3.6% and mean sensitivity was 18.0 (SD: 3.2) ng/ml. Inter-assay and intra-assay coefficients of variations were 13.7% and 3.0%, respectively.

Steller's eider

For the drive conducted in Izembek lagoon in 2001, three outliers (i.e., not within two standard deviations of the mean) above 145 ng/ml (155, 201, and 162 ng/ml) occurred between 182 and 193 minutes after the start of the drive and were deleted from further statistical analysis. Peak CORT mean level (88.4 ng/ml) was observed at 191-205 minutes after the boat drives were initiated (Fig. 2-2). The difference between the means was not found to be statistically different ($F = 2.13$, $P = 0.051$).

In 2003, the three drives overlapped slightly in time and formed a continuum from 43 to 255 min TSSD (drive # 1: 43 to 123 min TSSD; drive # 2: 108 to 185 min TSSD and drive # 3:

149 to 255 min TSSD). No significant relationship between time since capture and CORT concentrations was found (r = -0.220, P = 0.212; Fig. 2-3).

No significant differences were found between seasons (breeding and molting; F = 0.41, P = 0.528) or sexes (F = 3.08, P = 0.095) in captive STEI and no trend in these variables were observed (Table 2-2). Wild males had significantly higher CORT concentrations compared to captive males and females (F = 5.95, P = 0.001) but did not differ from wild females (Table 2-2.)

Spectacled eider and Pacific common eider

Mean CORT concentrations in samples from COEI breeding on the North Slope in 2001 were significantly lower (3-4 times) than COEI breeding in the same area in 2003 (q =13.102, P = 0.0001) or COEI from the Y-K Delta in 2003 (q = 8.809, P <0.001) (Table 2-3). A significant difference in mean CORT concentrations also was observed between captive female SPEI and wild con specifics from the Y-K Delta in 2002 (q = 3.802, P = 0.035) and 2003 (q = 2.448, P < 0.024). Captive birds had concentrations which were 3 to 4 times lower than those in the wild birds (Table 2-3). No significant difference in mean CORT concentrations for either season (breeding and molting) or sex was found among captive SPEI (Table 2-3). Comparisons between breeding females from different species showed no significant difference in means among COEI North Slope 2003, COEI Y-K Delta 2003, SPEI Y-K Delta 2002 and 2003 and no significant difference between COEI North Slope 2001 and captive SPEI females (Table 2-3).

King eider

The birds in the "post-op" group had significantly higher CORT concentrations than birds in the "anesthesia" group (q = 2.566, P = 0.043; Table 2-4). No statistical difference was observed between the other groups (Table 2-4).

DISCUSSION

Steller's eider

No clear trend in CORT concentrations in STEI involved in molting drives was observed. The mean CORT concentrations obtained from both the 2001 and the 2003 drives, 71.6 (±10.5) ng/ml and 72.0 (±3.1) ng/ml, are similar to those observed for birds sampled during breeding season, 81.0 (±11.9) ng/ml, on the North Slope of Alaska. At this time it is not known to what degree the capture procedure employed (i.e., boat based drive) acts as a stressor on wild STEI, however, a certain amount of stress resulting from the capture is likely.

The difference in mean CORT concentrations between wild and captive male STEI during the breeding time could be due to several factors, such as: i) variation in the degree of breeding activity/effort between wild and captive birds, ii) small sample size for wild birds, and iii) breeding site/ latitude differences (Romero *et al.*, 1998, Silverin and Wingfield; 1998). The lower overall CORT concentrations in the captive birds may be explained by habituation to human presence and handling, absence of predators and contaminants, and good nutritional health. Studies on turkeys have shown habituation in the CORT response over the course of several weeks when repeatedly exposed to different stressors (El Halawani *et al.*, 1973). The CORT concentrations in captive STEI are similar to concentrations observed in captive harlequin ducks, *Histrionicus histrionicus*, a species which has been proposed as a surrogate for STEI. The results suggest that in terms of baseline CORT concentrations these two species may be comparable.

Spectacled eider and Pacific common eider

Low CORT concentrations observed in COEI breeding on the North Slope in 2001 may be due to sample collection within 3 minutes of capture whereas all other COEI and SPEI

samples were gathered within approximately 3 – 10 minutes. Although this would indicate a very rapid stress response, many other studies have demonstrated the importance of rapid initial sampling to obtain baseline concentrations of CORT in a variety of bird species (white-crowned sparrow, *Zonotrichia leucophrys*, Astheimer *et al.*, 1994; snow buntings, *Plectrophenax nivalis*, Wingfield *et al.,* 1994a; redpolls, *Carduelis flammea* Romero *et al.*, 1998). In addition, inter-annual variation and different breeding sites have been shown to explain large differences in baseline CORT concentrations within a species during breeding time (redpolls, Romero *et al.*, 1998; pied flycatchers, *Ficedula hypoleuca*, Silverin and Wingfield, 1998; semi-palmated sandpipers, *Calidris pusilla*, Mizrahi *et al.*, 2001). The overall low concentrations observed in captive SPEI is probably a function of life in captivity, an abundance of food, and a lack of predators or contaminants. Capture, starvation or restricted food intake, low body mass, presence of a predator, and contaminants are all known to increase CORT concentrations in several species of wild birds (contaminants: Holmes *et al.*, 1979; capture: Gratto-Trevor *et al.*, 1991; Wingfield *et al.*, 1992; Wingfield *et al.*, 1994a; low body mass: Ramenofsky *et al.*, 1995; presence of a predator: Silverin, 1998b; starvation/low food intake: Mizrahi *et al.*, 2001; Cockrem and Silverin, 2002b;).

King eider

The body copes with a potentially life threatening situation with a temporary increase in circulating CORT concentrations (e.g., the stress response), thereby facilitating survival enhancing mechanisms, such as increased rates of gluconeogenesis (McEwan and Stellar, 1993; Wingfield *et al.*, 1998). The high CORT concentrations observed during the "post-op" stage may have been induced by handling and surgery. Lower concentrations in the king eiders considered

"recovered" may indicate the termination of a fairly brief stress response. However, all results from the present study were generated by small samples sizes.

The effects of anesthesia on stress hormone concentrations in these birds are unknown and may have influenced CORT concentrations in the king eiders. A study investigating the effects of anesthesia and surgery on CORT concentrations would be essential in identifying stressors during surgical procedures.

In conclusion, the RIA used in this study was validated to measure serum CORT concentrations in Steller's, spectacled, Pacific common, and king eiders. Captive Steller's and spectacled eiders showed overall lower CORT concentrations than wild con-specifics, which may be explained by an abundance of food, absence of predators and low parasite and disease loads in the captive birds. Breeding female Pacific common and spectacled eiders showed similar CORT concentrations. The reason(s) for the large variations among individuals in CORT concentrations observed in molting Steller's eiders during mass drives are unknown, but may include individual differences in responses to this type of stress and differences in molting stage. Studies on captive birds may prove useful in investigating these differences in a controlled setting.

As other studies have shown, rapid post-capture blood sampling is critical for evaluation of baseline corticosterone levels. Future studies investigating stress induced by surgical procedures, such as radio-transmitter implantation, on sea ducks may consider investigating the effects of anesthesia (e.g. dose, type, and time spent under anesthesia) on CORT concentrations. Opportunistic fecal sampling in conjunction with repeated blood sampling throughout the surgery may prove helpful in determining the level of stress induced by these types of procedures.

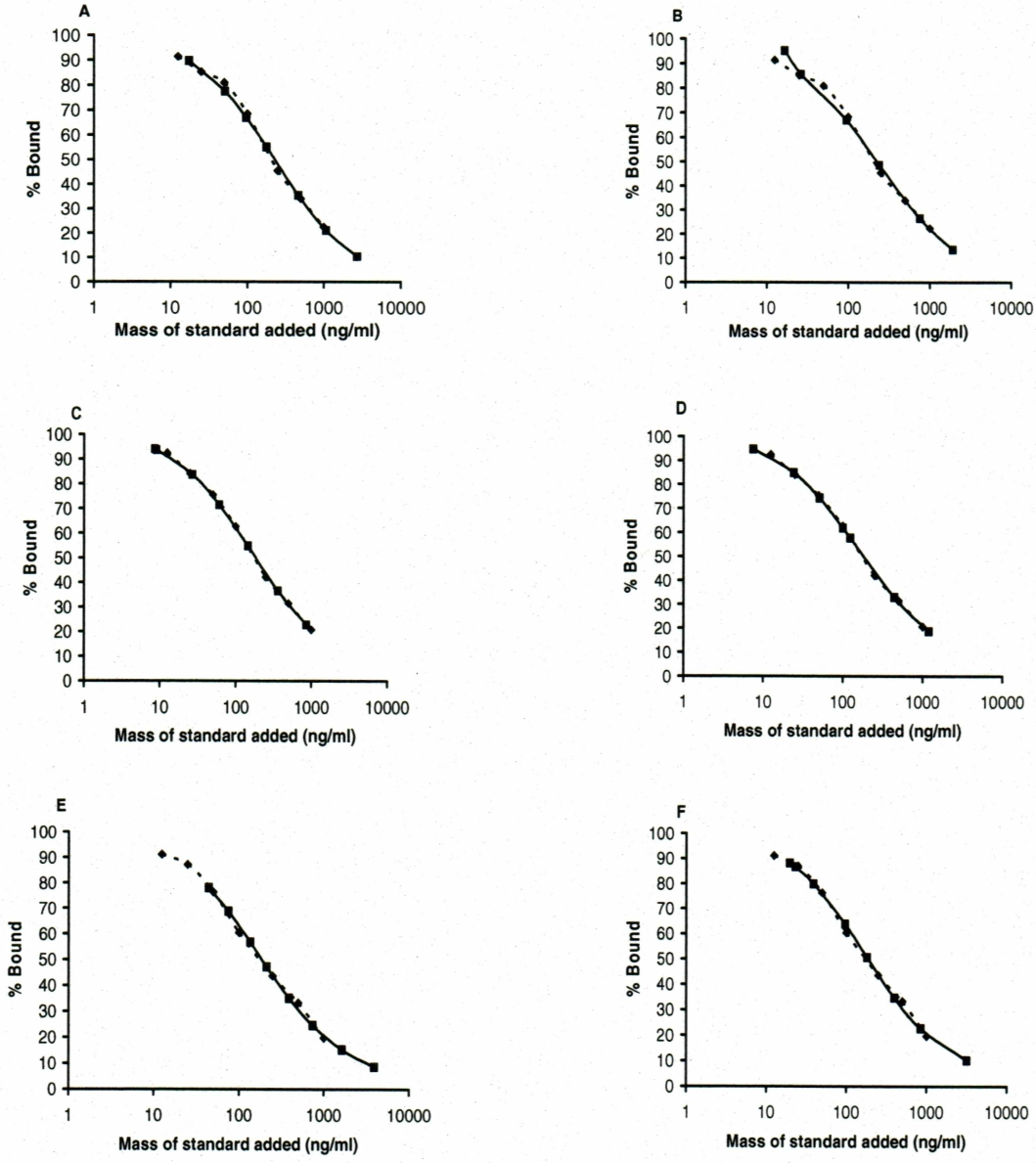

FIG. 2-1. Parallelism of a) female Steller's eider, b) male Steller's eider, c) female spectacled eider, d) female Pacific common eider, e) female king eider, and f) male king eider serum added to a corticosterone standard curve as compared to the plain radioimmunoassay standard curve. Curves of percent binding of ^{125}I - CORT versus serially diluted pooled eider serum (solid lines) were parallel to the standard curve (dashed lines) in all cases.

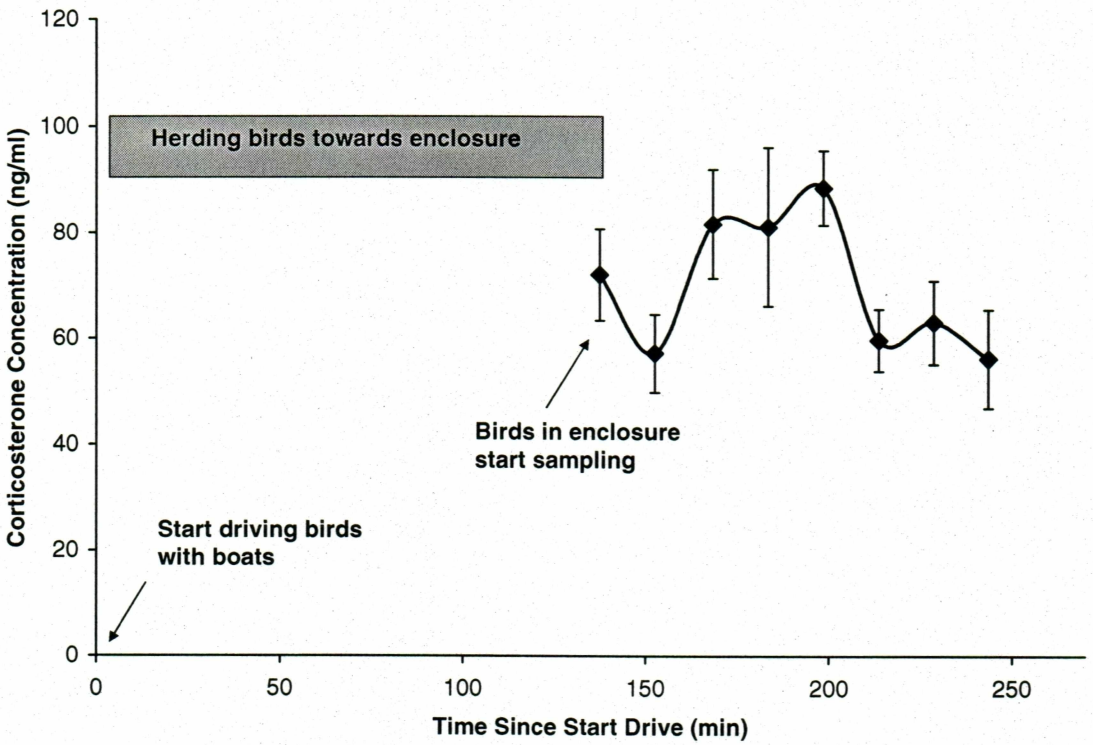

FIG. 2-2. Mean corticosterone concentrations for molting Steller's eiders captured in a mass drive at Izembek Lagoon, Alaska (55° 16' N; 162° 54' W) in 2001. "Time Since Start of Drive" refers to time elapsed since boats first approached eiders and started herding them towards the trap/enclosure.

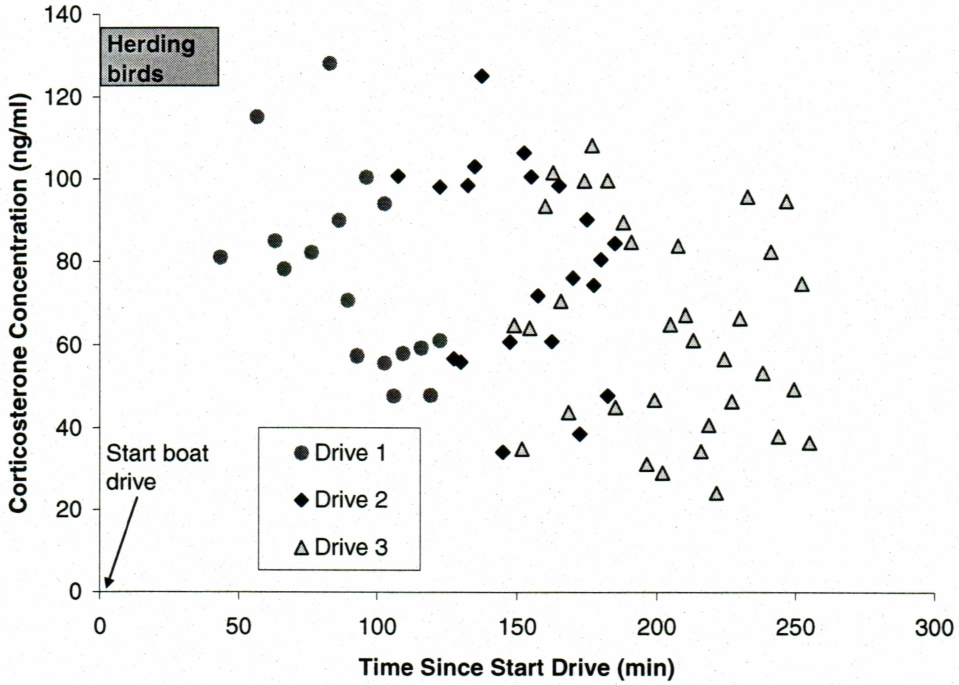

FIG. 2-3. Distribution of corticosterone concentrations over time since the start of the drive, for three mass drives of Steller's eiders undergoing flight feather molt in Izembek Lagoon, Alaska (55° 16' N; 162° 54' W) in 2003. "Time Since Start of Drive" refers to time elapsed since boats first approached eiders and started to herd them towards the trap/enclosure.

TABLE 2-1. Corticosterone radioimmunoassay accuracy check regression coefficients and mean recovery rate (%) for Steller's, spectacled, Pacific common, and king eiders.

Species	Sex	Regression slope	r^2	% Mean recovery rate
Steller's Eider	Female	1.014	0.990	84.2
Steller's Eider	Male	1.063	0.996	78.3
Spectacled Eider	Female	1.029	0.996	100.4
Pacific Common Eider	Female	1.063	0.999	88.8
King Eider	Male	1.057	0.980	106.4
King Eider	Female	1.040	0.952	81.5

Note: Regression analysis of eider serum added to a standard curve.

TABLE 2-2
Summary statistics of corticosterone concentrations for captive (breeding and molting seasons) and wild (breeding season) Steller's eiders

Status	Year	Season	Gender	*N*	Mean	Std Dev	Max	Min
Captive[1]	2003	Molting	M	6	32.7	13.0	57.5	21.3
Captive[1]	2003	Molting	F	5	55.3	24.4	85.0	20.9
Captive[1]	2003	Breeding	M	7	38.1	12.1	50.2	21.1
Captive[1]	2003	Breeding	F	3	37.7	15.2	55.3	28.6
Wild[2]	2001	Breeding	M	3	96.2	37.0	131.7	57.9
Wild[2]	2001	Breeding	F	3	66.0	8.6	75.8	59.8

Note: The molting eiders were at the end of primary flight feather molt at the time of sampling. [1]The captive birds were kept at the Alaska SeaLife Center, Seward, Alaska (60° 69' N; 149° 26' W). Average time between capture and bleed for captive eiders was 42 ± 4min (range: 10 to 78 min). [2]Eiders from the North Slope were caught near Barrow, Alaska (71° 17' N; 156° 47' W). Wild males differed significantly from captive males and females ($F = 5.95$, $P = 0.001$) but not from wild females. No significant statistical differences were detected between seasons or gender in the captive Steller's eiders.

TABLE 2-3

Summary statistics of corticosterone concentrations in female wild Pacific common eider (COEI) during breeding season, female wild spectacled eider (SPEI) during breeding season, and captive male and female SPEI during molting and breeding seasons.

Species	Site	Year	Season	Sex	*N*	Mean	Stdev	Max	Min
COEI	North Slope[1]	2001	Breed.	F	23	20.1	9.7	39.5	7.0
COEI	North Slope[1]	2003	Breed.	F	29	84.7	34.2	158.9	35.3
COEI	Y-K Delta[2]	2003	Breed.	F	22	68.0	21.9	121.1	23.3
SPEI	Y-K Delta[2]	2002	Breed.	F	27	90.7	35.5	171.5	30.5
SPEI	Y-K Delta[2]	2003	Breed.	F	20	67.0	30.4	120.2	18.6
SPEI	Captive ASLC[3]	2003	Molt.	M	6	14.9	7.8	28.8	7.2
SPEI	Captive ASLC[3]	2003	Molt.	F	6	14.9	7.1	25.0	6.0
SPEI	Captive ASLC[3]	2003	Breed.	M	6	20.7	14.1	42.9	5.1
SPEI	Captive ASLC[3]	2003	Breed.	F	5	21.1	13.2	40.8	8.2

Note: [1]Samples from breeding COEI females on the North Slope of Alaska were obtained from the vicinity of Prudhoe Bay (70° 20' N; 148° 21' W). [2]Breeding female COEI and SPEI were caught by the Kashunuk river (61° 20' N; 165° 35' W) in the Yukon-Kuskowkim Delta (Y-K Delta). [3]Captive spectacled eiders were housed at the Alaska SeaLife Center (ASLC), Seward, Alaska (60° 69' N; 149° 26' W). COEI breeding on the North Slope in 2001 were bled within 3 min of capture, all other wild samples (e.g. COEI North Slope 2003, COEI Y-K Delta 2003, SPEI Y-K Delta 2002 and 2003) were obtained within 10 min of capture. COEI North Slope. Mean time between capture and bleed for captive SPEI was 46±7 min (range 13 to 89 min). Mean CORT concentrations from COEI breeding on the North Slope in 2001 were significantly lower than COEI breeding in the same area in 2003 ($q = 13.102$, $P < 0.001$) or COEI from the YK Delta in 2003 ($q = 8.809$, $P = 0.001$). A significant difference in mean CORT concentrations was observed between captive female SPEI and wild conspecifics from the Y-K Delta in 2002 ($q = 3.802$, $P = 0.035$) and 2003 ($q = 2.448$, $P = 0.024$. Between-species comparisons for breeding females showed no significant differences in means among COEI NS 2003, COEI Y-K Delta 2003, SPEI Y-K Delta 2002 and 2003, and no significant difference between COEI NS 2001 and captive SPEI females. No significant differences were found between seasons or genders for the captive spectacled eiders.

TABLE 2-4
Summary statistics of corticosterone concentrations of king eiders undergoing radio-transmitter surgery.

Surgery Stage	*N*	Mean	Std Dev	Max	Min
Anesthesia	2	47.0	4.1	49.9	44.1
Post-op	5	110.4	34.9	162.0	81.2
Recovered	3	60.7	8.8	70.2	52.9

Note: The surgery procedure was performed in the field by the Kagloryuak river in the Canadian arctic (70° 16' N; 109° 59' W). Time between capture and surgery ranged between 3 and 9 hrs (mean: 5 hrs 48 min ± 36 min). Mean CORT concentrations differed significantly between surgery stage groups ($H = 7.6$, $df = 2$, $P = 0.002$); mean Post-op CORT concentration was significantly higher than the Anesthesia group's (Multiple comparison procedure, Dunn's method; $P = 0.043$). No statistical difference was observed among the other groups.

General Conclusions

The radioimmunoassay described herein is a valid and functional method to evaluate CORT concentrations in serum of the four species of eider ducks as well as in harlequin duck serum and feces. CORT is widely accepted as the major glucocorticoid metabolite in birds, which was re-confirmed for harlequin ducks in this study by performing HPLC.

As many other avian studies have shown, when collecting blood samples for stress hormone analysis it is critical that sampling is rapid and that the time of capture and subsequent samples are recorded, since the capture procedure itself is likely to induce stress.

Overall, it was found that captive harlequin ducks as well as spectacled and Steller's eiders display lower CORT concentrations compared to wild individuals. This observation could be explained by habituation of the birds to handling (i.e., the act of being "captured" is not perceived as a threat to the survival of the individual and hence does not elicit a strong stress response in captive birds) or potentially by captive birds being in overall better condition (low parasite/disease loads, good body condition, shelter from environmental stressors such as predators), a combination of the two, or some other unidentified factor(s).

Harlequin duck

The absence of a clear circadian pattern in CORT production in harlequin ducks sampled every four hours for a 24-hour period may have been a result of the time of year (spring) when the study was conducted. Some individuals may have more rapidly adjusted to the upcoming long arctic summer in terms of regulating hormone production, whereas others may "lag behind". It is recommended that future studies addressing similar research questions be conducted during both mid-summer and winter months.

Harlequin ducks showed a substantial increase in both serum and fecal CORT concentrations in response to an ACTH stimulation. The relative increase in CORT, as well as the time lag between ACTH injection and peak CORT concentrations for both serum and feces, were similar to those observed in other avian species (Spelman *et al.,* 1995, Wasser *et al.*, 1997; Ludders *et al.,* 2001; Goymann *et al.,* 2002). The results from this study suggest that fecal material can be used to investigate stress hormone concentrations in harlequin ducks as a non-invasive alternative to blood collection.

The negative correlation between PCV values and CORT concentrations in harlequin ducks suggests increased production of CORT as the body becomes progressively more anemic, or vice versa, or that some other factor affected both parameters.

Blood samples obtained within 17 minutes of capture of wild female harlequin ducks exhibited linearly increasing CORT concentrations with time since capture, as expected from results of other studies (Gratto-Trevor, 1991; Pravosudov, 2002; Silverin, 1997). The consistently high CORT concentrations observed in harlequin ducks which had been contained in transport kennels for several hours after capture suggest that as a stress stimulus continues (in this case, being held captive), CORT concentrations remain high. Further studies on the influence of short term captivity (i.e., hours) on CORT concentrations in sea ducks should consider utilizing fecal samples as a non-invasive method to evaluate those concentrations in addition to repeated serum sampling.

Samples from harlequin ducks inhabiting a region of Prince William Sound, Alaska where residual oil from the *Exxon Valdez* oil spill was still present several years post spill showed higher CORT concentrations during two consecutive years when compared to harlequin ducks inhabiting a region where no residual oil was found. However, the difference was not found to be statistically significant.

Steller's eider

CORT concentrations in eiders involved in mass drives varied widely between individuals and the interpretation of these concentrations are confounded by several factors. The importance of each of these factors as a potential cause of stress is hard to tease apart and in turn gives rise to additional questions. For example, are the eiders reacting at all on a hormonal level to the drive and capture procedure? Is it the drive or the actual "capture" that is perceived as the main stressor, if indeed, the eiders do show a stress response? Do individual drives differ in their potential to act as a stress stimulus and, if so, how much and what do these differences depend on? What is the influence of the stage of molt on CORT concentrations? Sampling of captive birds may prove a useful tool for investigating seasonal differences as well as differences in CORT concentrations during different stages of molt. Results from this study suggest that baseline CORT concentrations are comparable between captive Steller's eiders and harlequin duck.

Spectacled eider and Pacific common eider

Samples from breeding female spectacled eiders and Pacific common eiders showed similar overall concentrations of CORT suggesting that these two species are comparable in terms of baseline CORT concentrations. The large difference in CORT concentrations observed between a year when common eider females were bled within three minutes and all other years for common eiders and spectacled eiders when the bleeds were conducted < 10 minutes post capture likely elucidates the quick stress response in these birds and the importance of rapid sampling.

King eider

In order to thoroughly investigate stress induced by surgical procedures and radio-transmitter implantation on sea ducks, several variables need to be investigated. For example, the influence of anesthesia on CORT concentrations (e.g., type, dose, time spent under anesthesia), and total time spent in captivity before surgery. Repeated blood sampling throughout the entire procedure (i.e., from capture, through transport, holding time, surgery, post-op, and pre-release) as well as opportunistic fecal sampling will be essential to answer these questions.

Overall conclusions

In conclusion, radioimmunoassay techniques utilizing commercially available RIA-kits can be used to establish CORT concentrations in eiders and harlequin ducks. Information on CORT concentrations in individuals and populations of sea ducks could provide a useful tool for assessing the health and trends of these populations, and may be helpful to wildlife managers and policy makers in making informed decisions by increasing understanding and recognition of populations undergoing stress.

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